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(54) Title: MORPHOGEN-INDUCED PERIODONTAL TISSUE REGENERATION (57) Abstract <p>Disclosed are methods and compositions for inducing periodontal tissue morphogenesis in a mammal which include a therapeutically effective concentration of a morphogen. The methods and compositions are useful for integrating an implanted tooth in a tooth socket and for inhibiting tissue loss associated with periodontal disease or injury.</p>		

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MORPHOGEN-INDUCED PERIODONTAL TISSUE REGENERATION

Background of the Invention

This invention relates generally to the dental arts and
5 more specifically to methods and compositions for treating
and regenerating periodontal tissue.

The peridontium is the cushioning tissue which anchors
the tooth root to the mandibular or maxillar jawbone tissue
10 by suspending the tooth in the tooth socket ("alveolus").
Periodontal tissue includes both the periodontal ligament, a
collagen-containing tissue that is in contact with the bone
tissue, and cementum, a mineralized tissue that covers the
dental root surface. These two hard tissues are connected
15 through the periodontal ligament fibers that run in a
perpendicular direction to the two surfaces and thereby
serve to anchor and suspend the tooth in the tooth socket,
providing a shock-absorptive cushion between the tooth and
the jawbone that accommodates the pressure applied to teeth
20 when food is being chewed.

Periodontal tissue loss may occur as a result of
disease, including infectious diseases (e.g., gingivitis,
caused by bacteria), nutritional diseases, e.g., scurvy,
25 resulting from a vitamin deficiency, and a number of
neoplastic diseases, including acute leukemia and lymphomas.
The diseases are characterized by inflammation, bleeding and
ulceration. Periodontal disease also may result from an
opportunistic infection, e.g., in an immune-compromised
30 individual. Left untreated, these diseases can cause
significant periodontal tissue loss which loosen the tooth
and ultimately can result in loss of the tooth and the
alveolar bone tissue (periodontitis.) Chronic periodontitis
is the primary cause of tooth loss in adults. Current
35 treatments include professional cleaning to remove plaque

and tartar, use of oral antiseptics, local and/or systemic antibiotic therapies, and/or surgical procedures to remove periodontal pockets formed from periodontal tissue lesions and necrosis. Typically, where a tooth has been lost as a
5 result of periodontitis, a prosthetic tooth or removable bridge is substituted for the natural tooth.

Periodontal tissue loss also may occur as a result of mechanical injury to the tissue or to the tooth itself,
10 particularly one causing tooth loss. Tooth loss also may occur as a result of any of a number of dental diseases, e.g., dental caries, pulpitis, or osteomyelitis.

A viable tooth can be reimplanted if implantation occurs
15 quickly after loss, e.g., within thirty minutes, and if the periodontal tissue within the tooth socket is still healthy. However, if a significant period of time is allowed to elapse, the living periodontal tissue lining the tooth socket will be resorbed. In addition, the tooth itself
20 begins to degenerate and a prosthetic tooth or removable bridge must be implanted. In the absence of healthy periodontal tissue the prosthetic implant is integrated directly into the jaw bone tissue in a condition called ankylosis (bone tissue in direct contact with dentin
25 tissue.) The life of such prosthetic tooth implants often is limited due to the absence of viable periodontal tissue to enhance tooth anchoring and to absorb the impact of mastication on the prosthesis.

30 It is an object of this invention to provide a means for inhibiting periodontal tissue loss, as well as means for inducing regeneration of damaged periodontal tissue. Another object is to provide means for inhibiting the periodontal tissue damage and tooth loss associated with
35 periodontal and other gum diseases. Yet another object is

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to enhance integration of an implanted tooth, including a reimplanted natural tooth or tooth prosthesis, in the tooth socket. Still another object is to promote periodontal tissue growth around an implanted tooth. Another object is
5 to inhibit ankylosis of an implanted tooth or tooth prosthesis.

These and other objects and features of the invention will be obvious from the specification, drawings and claims,
10 which follow.

Summary of the Invention

The invention provides methods and compositions for inhibiting periodontal tissue loss in a mammal, particularly humans, including regenerating damaged tissue and/or inhibiting additional damage thereto. The methods and compositions of this invention may be used to prevent and/or inhibit tooth loss, as well as to enhance integration of an implanted tooth.

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As used herein, "implanted tooth" includes a natural tooth which has grown naturally in the tooth socket, a natural tooth which is reimplanted in a tooth socket, and a prosthetic tooth, which includes both natural teeth from which the root has been removed and replaced with an inert, biocompatible material, and "complete" prostheses made of natural or synthetic, non dentin-containing materials. In all cases, "tooth" refers to a natural or synthetic composition essentially defining the shape of a natural tooth, having a solid tooth body, including a crown and tooth root. "Reimplanted natural tooth" includes both an allogenic tooth, e.g., selected from a tooth bank; and a tooth autologous to the mammal, such as a tooth which has fallen out, been knocked out, or otherwise removed from the individual into which it is now being reimplanted.

"Integrated tooth" means an implanted tooth with a living, substantially healthy periodontal tissue, including periodontal ligament and cementum, anchoring the tooth to the jaw bone. "Viable" tissue means living, substantially healthy tissue. "Viable tooth" refers to an implanted natural tooth with a living tooth root. "Periodontium" defines the tissues which surround the tooth in the tooth socket and includes both periodontal ligament and cementum. "Inhibit loss" of periodontal tissue, as used herein, means inhibiting damage to, and/or loss of, periodontal tissue,

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- 5 -

including periodontal ligament and/or cementum, and includes regenerating lost or damaged tissue and/or inhibiting additional damage thereto. "Symptom alleviating cofactor" refers to one or more pharmaceuticals which may be

5 administered together with the therapeutic agents of this invention and which alleviate or mitigate one or more of the symptoms typically associated with periodontal tissue loss. Exemplary cofactors include antibiotics, antiseptics, non-steroidal antiinflammatory agents, anaesthetics and

10 analgesics.

The methods and compositions of this invention include a morphogenic protein ("morphogen"), as described herein, which, when provided to the tooth and/or jawbone surfaces in

15 a tooth socket is capable of inducing periodontal tissue formation where periodontal tissue has been lost or damaged, and enhancing integration of an implanted tooth thereby.

In one aspect, the invention features therapeutic

20 treatment methods and compositions for inhibiting periodontal tissue loss in a mammal which include administering to the individual a therapeutically effective morphogen at a concentration and for a time sufficient to regenerate damaged periodontal tissue and/or to inhibit

25 additional damage thereto.

In another aspect, the invention features therapeutic treatment methods and compositions for inhibiting periodontal tissue loss in a mammal which include

30 administering to the individual a compound that stimulates in vivo a therapeutically effective concentration of an endogenous morphogen in the body of the mammal sufficient to regenerate damaged periodontal tissue and/or to inhibit additional damage thereto. These compounds are referred to

35 herein as morphogen-stimulating agents, and are understood

to include substances which, when administered to a mammal, act on cells of tissue(s) or organ(s) that normally are responsible for, or capable of, producing a morphogen and/or secreting a morphogen, and which cause the endogenous level
5 of the morphogen to be altered. The agent may act, for example, by stimulating expression and/or secretion of an endogenous morphogen. In preferred embodiments, the agent stimulates expression and/or secretion of an endogenous morphogen so as to increase amounts of the morphogen in the
10 alveolar bone, periodontium or cementum tissue cells.

In another aspect, the invention provides methods and compositions for enhancing the integration of an implanted tooth, particularly where the tooth socket is substantially
15 reduced in viable periodontal tissue. In fact, the processes and compositions of the invention work well when a tooth socket has lost 30-50% of the periodontal ligament, and as much as 50-100% of the periodontal ligament. The methods and compositions include providing to the tooth or
20 tooth socket surface a therapeutically effective concentration of a morphogen or morphogen-stimulating agent sufficient to induce morphogenesis of periodontal tissue. The implanted tooth may be an implanted tooth which has grown naturally in the socket and which is loose as a result
25 of, for example, mechanical injury or due to a dental or periodontal disease. Alternatively, the implanted tooth may be a lost tooth or a tooth prosthesis which has been reimplanted in a vacant tooth socket. The tooth prosthesis may include a natural tooth from which a damaged or diseased
30 root has been removed and replaced with a biocompatible, biologically inert material, as is created in a root canal procedure. The prosthetic tooth also may be composed of synthetic, non dentin-containing materials.

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The morphogen may be provided directly to the tooth surface to be implanted, and/or to the tooth socket to which the tooth is to be implanted. Where the morphogen is to be provided to the tissue socket, it may be provided by topical administration to the tooth socket surface or by local injection to periodontal or alveolar bone tissue associated with the socket. Alternatively, an agent capable of stimulating the production and/or secretion of a therapeutically effective concentration of an endogenous morphogen also may be provided to the tooth or tooth socket. Where the morphogen or morphogen stimulating agent (referred to herein collectively as "therapeutic agent") is provided to the tooth surface, it preferably is dispersed in a biocompatible, bioresorbable carrier, most preferably a carrier capable of retaining the therapeutic agent at the tissue surface and/or providing a controlled delivery of the agent to the tooth socket. The therapeutic agent also may be provided to the tooth socket itself, also preferably in association with a carrier capable of maintaining the agent in the tooth socket, and/or capable of enhancing the controlled delivery of the agent to the socket. Useful carriers include compositions having a high viscosity, such as that provided by glycerol and the like, as well as carrier materials formulated from extracellular matrices and/or which contain laminin, collagen, and/or biocompatible synthetic polymers, such as polybutyric, polylactic, polyglycolic acids and copolymers thereof. In addition, or alternatively, an acellular carrier material may be formulated from bone, dentin, cementum or periodontal tissue by demineralizing and guanidine-extracting the tissue essentially as described herein and/or in international application US92/01968 (WO92/15323). Particularly useful acellular matrices include dentin-derived, periodontal ligament-derived and cementum-derived matrices.

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In addition, the tooth to be implanted preferably comprises a porous exterior surface onto which the therapeutic agent may be adsorbed, and into which progenitor and differentiating cementoblasts can infiltrate and proliferate. Useful surfaces include natural tooth root surfaces, and porous prosthetic surfaces, including surfaces composed of matrix materials such as collagen, laminin, biocompatible polymers or metals such as titanium oxide. Where a natural tooth or dentin-containing prosthesis is to be implanted, the surface to be implanted first may be partially demineralized, e.g., by transient exposure to an acid to enhance the porosity of the tooth root surface.

Preferably, where the tooth is to be implanted into a tooth socket, the socket has been freed of fibrous tissue which may have formed following tooth loss and periodontal tissue resorption. For example, the tooth socket may have undergone a healing period of several months after loss or removal of the tooth such that scar tissue has formed over the wound. In this case the healed socket preferably is surgically prepared for tooth implantation by removing the scar and other undesired tissue to expose the alveolar bone surface.

Preferably, where the therapeutic agent is to be provided to enhance periodontal tissue viability surrounding an implanted tooth, the therapeutic agent is provided topically to the tissue surfaces between the tooth and gingiva. Alternatively, the agent may be injected locally, e.g., into the gingiva itself.

The morphogens described herein may be used to inhibit periodontal tissue loss and/or to enhance viability of periodontal tissue at risk of damage due to a periodontal disease. The periodontal disease may be caused by an

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infectious agent, such as a bacterial, fungal or viral agent, or by a nutritional deficiency, including a vitamin deficiency. The morphogens also may be used to regenerate periodontal tissue lost as a result of a neoplastic disease, including squamous cell carcinomas, acute leukemias, lymphomas and metastatic tumors. A detailed description of diseases which damage or destroy periodontal tissue can be found, for example, in Harrison's Principles of Internal Medicine, 243-248, (McGraw-Hill 12th ed. 1991), the disclosure of which is incorporated herein by reference. The efficacy of the morphogens described herein in modulating an inflammatory response are described in detail in international application US92/07358 (WO93/04692).

Although all individuals, and particularly adults, are at risk for periodontal tissue damage due to periodontal disease, a population most particularly at risk are immune-compromised individuals, such as individuals suffering from autoimmune diseases and/or whose immune system has been suppressed as part of a clinical procedure or therapy. Thus, in another aspect, the invention provides methods and compositions for inhibiting periodontal tissue loss in immune-compromised individuals.

As described in international application WO92/15323, and Example 2, below, the morphogens described herein also can induce formation of damaged or lost dentin tissue. Accordingly, where a natural tooth or dentin-containing prosthesis is to be implanted, a morphogen or morphogen-stimulating agent also may be provided to damaged areas of the tooth to induce dentin regeneration of damaged or lost dentin tissue. The morphogen may be provided topically or otherwise administered to the tooth tissue. For example, the morphogen may be dispersed in a biocompatible, porous carrier material that then is provided topically to the

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damaged dentin tissue. A useful carrier may be formulated from dentin by demineralizing and guanidine-extracting the tissue to create an acellular matrix.

5 The morphogens and morphogen-stimulating agents also may be provided to the periodontium together with other molecules ("cofactors") known to have a beneficial effect in treating damaged periodontal tissue, particularly cofactors capable of mitigating or alleviating symptoms typically
10 associated with periodontal tissue damage and/or loss. Examples of such cofactors include antiseptics such as chlorohexidine and tibezoneum iodide, antibiotics, including tetracycline, aminoglycosides, macrolides, penicillins and cephalosporins, anaesthetics and analgesics, and other non-
15 steroidal anti-inflammatory agents.

 Among the morphogens useful in this invention are proteins originally identified as osteogenic proteins (see U.S. Patent 5,011,691, incorporated herein by reference),
20 such as the OP-1, OP-2 and CBMP2 proteins, as well as amino acid sequence-related proteins such as DPP (from Drosophila), Vgl (from Xenopus), Vgr-1 (from mouse), GDF-1 (from mouse, see Lee (1991) PNAS 88:4250-4254), all of which are presented in Table II and Seq. ID Nos. 5-14, and the
25 recently identified 60A protein (from Drosophila, Seq. ID No. 24, see Wharton et al. (1991) PNAS 88:9214-9218.) The members of this family, which include members of the TGF- β super-family of proteins, share substantial amino acid sequence homology in their C-terminal regions. The proteins
30 are translated as a precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature sequence. The "pro" form of the protein includes the pro domain and the mature domain, and forms a soluble
35 species that appears to be the primary form secreted from

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cultured mammalian cells. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne ((1986) Nucleic Acids Research 14:4683-4691.) Table I, below, describes the various morphogens identified to date, including their nomenclature as used herein, their Seq. ID references, and publication sources for the amino acid sequences for the full length proteins not included in the Seq. Listing. The disclosure of these publications is incorporated herein by reference.

TABLE I

15	"OP-1"	Refers generically to the group of morphogenically active proteins expressed from part or all of a DNA sequence encoding OP-1 protein, including allelic and species variants thereof, e.g., human OP-1 ("hOP-1", Seq. ID No. 5, mature protein amino acid sequence), or mouse OP-1 ("mOP-1", Seq. ID No. 6, mature protein amino acid sequence.) The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 5 and 6. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 16 and 17 (hOP1) and Seq. ID Nos. 18 and 19 (mOP1.) The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).
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"OP-2"

Refers generically to the group of active proteins expressed from part or all of a DNA sequence encoding OP-2 protein, including allelic and species variants thereof, e.g., human OP-2 ("hOP-2", Seq. ID No. 7, mature protein amino acid sequence) or mouse OP-2 ("mOP-2", Seq. ID No. 8, mature protein amino acid sequence). The conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 7 and 8. The cDNA sequences and the amino acids encoding the full length proteins are provided in Seq. ID Nos. 20 and 21 (hOP2) and Seq. ID Nos. 22 and 23 (mOP2.) The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP1).

"CBMP2"

Refers generically to the morphogenically active proteins expressed from a DNA sequence encoding the CBMP2 proteins, including allelic and species variants thereof, e.g., human CBMP2A ("CBMP2A(fx)", Seq ID No. 9) or human CBMP2B DNA ("CBMP2B(fx)", Seq. ID No. 10). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988) Science 242:1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248; the mature protein, residues 249-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256; the mature protein, residues 257-408.

- 5 "DPP(fx)" refers to protein sequences encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Padgett, et al (1987) Nature 325: 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.
- 10 "Vgl(fx)" refers to protein sequences encoded by the Xenopus Vgl gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in Weeks (1987) Cell 51: 861-867. The prodomain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.
- 20 "Vgr-1(fx)" refers to protein sequences encoded by the murine Vgr-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13). The amino acid sequence for the full length protein appears in Lyons, et al, (1989) PNAS 86: 4554-4558. The prodomain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.
- 30 "GDF-1(fx)" refers to protein sequences encoded by the human GDF-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 14). The cDNA and encoded amino sequence for the full length protein is provided in Seq. ID. No. 32.
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The prodomain likely extends from the signal peptide cleavage site to residue 214; the mature protein likely is defined by residues 215-372.

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"60A" refers generically to the morphogenically active proteins expressed from part or all of a DNA sequence (from the Drosophila 60A gene) encoding the 60A proteins (see Seq. ID No. 24
10 wherein the cDNA and encoded amino acid sequence for the full length protein is provided). "60A(fx)" refers to the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of Seq. ID No.
15 24.) The prodomain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455.
- 20 "BMP3(fx)" refers to protein sequences encoded by the human BMP3 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 26). The amino acid sequence for the full length protein appears in Wozney et al. (1988) Science 242:
25 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.
- 30 "BMP5(fx)" refers to protein sequences encoded by the human BMP5 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 27). The amino acid sequence for the full length protein appears in Celeste, et al. (1991) PNAS 87:
35 9843-9847. The pro domain likely extends from

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the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.

5 "BMP6(fx)" refers to protein sequences encoded by the human BMP6 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 28). The amino acid sequence for the full length protein appears in Celeste, et al. (1990) PNAS 87: 9843-5847. The pro domain likely includes
10 extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.

15 The OP-2 proteins have an additional cysteine residue in the conserved region (e.g., see residue 41 of Seq. ID Nos. 7 and 8), in addition to the conserved cysteine skeleton in common with the other proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved
20 skeleton (residues 44-47 of Seq. ID No. 14) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. In addition, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton.

25 The morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention. Thus, as defined herein, a morphogen is a dimeric protein comprising a pair
30 of polypeptide chains, wherein each polypeptide chain comprises at least the C-terminal six cysteine skeleton defined by residues 43-139 of Seq. ID No. 5, including functionally equivalent arrangements of these cysteines (e.g., amino acid insertions or deletions which alter the
35 linear arrangement of the cysteines in the sequence but not

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th ir relationship in the folded structure), such that, when the polypeptide chains are folded, the dimeric protein species comprising the pair of polypeptide chains has the appropriate three-dimensional structure, including the appropriate intra- and/or inter-chain disulfide bonds such that the protein is capable of acting as a morphogen as defined herein. Specifically, the morphogens generally are capable of all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells. In addition, it is also anticipated that these morphogens are capable of inducing redifferentiation of committed cells under appropriate environmental conditions.

In one preferred aspect, the morphogens of this invention comprise one of two species of generic amino acid sequences: Generic Sequence 1 (Seq. ID No. 1) or Generic Sequence 2 (Seq. ID No. 2); where each Xaa indicates one of the 20 naturally-occurring L-isomer, α -amino acids or a derivative thereof. Generic Sequence 1 comprises the conserved six cysteine skeleton and Generic Sequence 2 comprises the conserved six cysteine skeleton plus the additional cysteine identified in OP-2 (see residue 36, Seq. ID No. 2). In another preferred aspect, these sequences further comprise the following additional sequence at their N-terminus:

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Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)

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Preferred amino acid sequences within the foregoing generic sequences include: Generic Sequence 3 (Seq. ID No. 3), Generic Sequence 4 (Seq. ID No. 4), Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31),
5 listed below. These Generic Sequences accommodate the homologies shared among the various preferred members of this morphogen family identified in Table II, as well as the amino acid sequence variation among them. Generic Sequences 3 and 4 are composite amino acid sequences of the proteins
10 presented in Table II and identified in Seq. ID Nos. 5-14, specifically: human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq.
15 ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14.) The generic sequences include both the amino acid identity shared by the sequences in Table II, as well as alternative residues for the variable positions within the
20 sequence. Note that these generic sequences allow for an additional cysteine at position 41 or 46 in Generic Sequences 3 or 4, respectively, providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids
25 which influence the tertiary structure of the proteins.

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Generic Sequence 3

Leu Tyr Val Xaa Phe

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Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa

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Xaa Ala Pro Xaa Gly Xaa Xaa Ala

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Xaa Tyr Cys Xaa Gly Xaa Cys Xaa

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Xaa Pro Xaa Xaa Xaa Xaa Xaa

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Xaa Xaa Xaa Asn His Ala Xaa Xaa

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Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys

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Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa

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Xaa Xaa Xaa Leu Xaa Xaa Xaa

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Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

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Xaa Xaa Xaa Xaa Met Xaa Val Xaa

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Xaa Cys Gly Cys Xaa

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wherein each Xaa is independently selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser or Lys); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu or Val); Xaa at res.11 = (Gln, Leu, Asp, His or Asn); Xaa at res.12 = (Asp, Arg or Asn); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Leu or Gln); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp or Gln); Xaa at res.28 = (Glu, Lys, Asp or Gln); Xaa at res.30 = (Ala, Ser, Pro or Gln); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu or Val); Xaa at res.34 = (Asn, Asp, Ala or Thr); Xaa at res.35 = (Ser, Asp, Glu, Leu or Ala); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn or Ser); Xaa at res.39 = (Ala, Ser or Gly); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile or Val); Xaa at res.45 = (Val or Leu); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His or Asn); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala or Val); Xaa at res.53 = (Asn, Lys, Ala or Glu); Xaa at res.54 = (Pro or Ser); Xaa at res.55 = (Glu, Asp, Asn, or Gly); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser or Ala); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys or Leu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr or Ala); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser or Asp); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr or Val); Xaa at

	Cys	Xaa	Xaa	Xaa	Xaa	Leu	Tyr	Val	Xaa	Phe
	1					5				10
20	Xaa	Xaa	Xaa	Gly	Trp	Xaa	Xaa	Trp	Xaa	
					15					
	Xaa	Ala	Pro	Xaa	Gly	Xaa	Xaa	Ala		
	20					25				
	Xaa	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa		
			30					35		
25	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa			
					40					
	Xaa	Xaa	Xaa	Asn	His	Ala	Xaa	Xaa		
			45					50		
	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa	Xaa		
30					55					
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys		
	60						65			
	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa		
					70					
35	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa			
	75					80				

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Xaa Xaa Xaa Xaa Val Xaa Leu Xaa

85

Xaa Xaa Xaa Xaa Met Xaa Val Xaa

90

95

5 Xaa Cys Gly Cys Xaa

100

wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 =

10 (Lys or Arg); Xaa at res.3 = (Lys or Arg); Xaa at res.4 = (His or Arg); Xaa at res.5 = (Glu, Ser, His, Gly, Arg or Pro); Xaa at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg, Gln, Ser or Lys); Xaa at res.12 = (Asp or Glu); Xaa at res.13 = (Leu or Val); Xaa at res.16 =

15 (Gln, Leu, Asp, His or Asn); Xaa at res.17 = (Asp, Arg, or Asn); Xaa at res.19 = (Ile or Val); Xaa at res.20 = (Ile or Val); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.25 = (Tyr or Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Leu, or Gln); Xaa at res.28 =

20 (Tyr, Asn or Phe); Xaa at res.31 = (Glu, His, Tyr, Asp or Gln); Xaa at res.33 = Glu, Lys, Asp or Gln); Xaa at res.35 = (Ala, Ser or Pro); Xaa at res.36 = (Phe, Leu or Tyr); Xaa at res.38 = (Leu or Val); Xaa at res.39 = (Asn, Asp, Ala or Thr); Xaa at res.40 = (Ser, Asp, Glu,

25 Leu or Ala); Xaa at res.41 = (Tyr, Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly or Leu); Xaa at res.44 = (Ala, Ser or Gly); Xaa at res.45 = (Thr, Leu or Ser); Xaa at res.49 = (Ile or Val); Xaa at res.50 = (Val or Leu); Xaa at res.51 = (Gln or Arg); Xaa at

30 res.52 = (Thr, Ala or Ser); Xaa at res.54 = (Val or Met); Xaa at res.55 = (His or Asn); Xaa at res.56 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn, Ala or Val); Xaa at res.58 = (Asn, Lys, Ala or Glu); Xaa at res.59 = (Pro or Ser); Xaa at res.60 =

35 (Glu, Asp, or Gly); Xaa at res.61 = (Thr, Ala, Val,

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Lys, Asp, Tyr, Ser or Ala); Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro or Asp); Xaa at res.64 = (Lys or Leu); Xaa at res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val); Xaa at res.70 = (Thr or Ala);

5 Xaa at res.71 = (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or Val); Xaa at res.73 = (Asn, Ser or Asp); Xaa at res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr or Val); Xaa at res.76 = (Ser or Ala); Xaa at res.77 = (Val or Met); Xaa at res.79 = (Tyr or Phe);

10 Xaa at res.80 = (Phe, Tyr or Leu); Xaa at res.81 = (Asp or Asn); Xaa at res.82 = (Asp, Glu, Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn or Tyr); Xaa at res.84 = (Ser, Asn, Asp or Glu); Xaa at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile or Val); Xaa at res.89 = (Lys or

15 Arg); Xaa at res.90 = (Lys, Asn, Gln or His); Xaa at res.91 = (Tyr or His); Xaa at res.92 = (Arg, Gln or Glu); Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 = (Val, Thr or Ala); Xaa at res.97 = (Arg, Lys, Val, Asp or Glu); Xaa at res.98 = (Ala, Gly or Glu); and Xaa

20 at res.102 = (His or Arg).

Similarly, Generic Sequence 5 (Seq. ID No. 30) and Generic Sequence 6 (Seq. ID No. 31) accommodate the homologies shared among all the morphogen protein

25 family members identified in Table II. Specifically, Generic Sequences 5 and 6 are composite amino acid sequences of human OP-1 (hOP-1, Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-22),

30 CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq. ID No. 13), and GDF-1 (from mouse, Seq. ID No. 14), human BMP3 (Seq. ID No. 26), human BMP5 (Seq. ID No. 27), human

35 BMP6 (Seq. ID No. 28) and 60(A) (from Drosophila, Seq.

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ID Nos. 24-25). The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine skeletons (Generic Sequences 5 and 6, respectively), as well as alternative residues for the variable positions within the sequence. As for Generic Sequences 3 and 4, Generic Sequences 5 and 6 allow for an additional cysteine at position 41 (Generic Sequence 5) or position 46 (Generic Sequence 6), providing an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and containing certain critical amino acids which influence the tertiary structure of the proteins.

15 Generic Sequence 5

```

                Leu Xaa Xaa Xaa Phe
                   1               5
Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa
20                   10
Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala
                   15               20
Xaa Tyr Cys Xaa Gly Xaa Cys Xaa
                   25               30
25 Xaa Pro Xaa Xaa Xaa Xaa Xaa
                   35
Xaa Xaa Xaa Asn His Ala Xaa Xaa
                   40               45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
30                   50

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys
 55 60
 Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa
 65
 5 Xaa Xaa Xaa Leu Xaa Xaa Xaa
 70 75
 Xaa Xaa Xaa Xaa Val Xaa Leu Xaa
 80
 Xaa Xaa Xaa Xaa Met Xaa Val Xaa
 10 85 90
 Xaa Cys Xaa Cys Xaa
 95

wherein each Xaa is independently selected from a group
 of one or more specified amino acids defined as
 15 follows: "Res." means "residue" and Xaa at res.2 =
 (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4
 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys
 or Ala); Xaa at res.7 = (Asp, Glu or Lys); Xaa at res.8
 = (Leu, Val or Ile); Xaa at res.11 = (Gln, Leu, Asp,
 20 His, Asn or Ser); Xaa at res.12 = (Asp, Arg, Asn or
 Glu); Xaa at res.14 = (Ile or Val); Xaa at res.15 =
 (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18
 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 =
 (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at
 25 res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly);
 Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 =
 (Glu, His, Tyr, Asp, Gln or Ser); Xaa at res.28 = (Glu,
 Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro,
 Gln or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at
 30 res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp,
 Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu,

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Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser, Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at
5 res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val);
10 Xaa at res.52 = (Ile, Met, Asn, Ala, Val or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His); Xaa at res.57 =
15 (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res.67 = (Leu, Met or Val); Xaa at res.68
20 = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Met or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or
25 Leu); Xaa at res.77 = (Asp, Glu, Asn or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln,
30 His or Val); Xaa at res.86 = (Tyr or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly
35 or Ala) and Xaa at res.97 = (His or Arg).

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Generic Sequence 6

	Cys	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Phe
	1					5				10
5	Xaa	Xaa	Xaa	Gly	Trp	Xaa	Xaa	Trp	Xaa	
				15						
	Xaa	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Ala		
	20					25				
	Xaa	Tyr	Cys	Xaa	Gly	Xaa	Cys	Xaa		
10			30					35		
	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa			
				40						
	Xaa	Xaa	Xaa	Asn	His	Ala	Xaa	Xaa		
			45					50		
15	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa		
				55						
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Cys		
			60					65		
	Cys	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa		
20				70						
	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa			
			75				80			
	Xaa	Xaa	Xaa	Xaa	Val	Xaa	Leu	Xaa		
				85						
25	Xaa	Xaa	Xaa	Xaa	Met	Xaa	Val	Xaa		
			90					95		
	Xaa	Cys	Xaa	Cys	Xaa					
				100						

30 wherein each Xaa is independently selected from a group of one or more specified amino acids as defined by the following: "Res." means "residue" and Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); Xaa at res.5 =

35 (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr); Xaa at

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res.7 = (Tyr or Lys); Xaa at res.8 = (Val or Ile); Xaa
at res.9 = (Ser, Asp or Glu); Xaa at res.11 = (Arg,
Gln, Ser, Lys or Ala); Xaa at res.12 = (Asp, Glu, or
Lys); Xaa at res.13 = (Leu, Val or Ile); Xaa at res.16
5 = (Gln, Leu, Asp, His, Asn or Ser); Xaa at res.17 =
(Asp, Arg, Asn or Glu); Xaa at res.19 = (Ile or Val);
Xaa at res.20 = (Ile or Val); Xaa at res.21 = (Ala or
Ser); Xaa at res.23 = (Glu, Gln, Leu, Lys, Pro or Arg);
Xaa at res.24 = (Gly or Ser); Xaa at res.25 = (Tyr or
10 Phe); Xaa at res.26 = (Ala, Ser, Asp, Met, His, Gln,
Leu, or Gly); Xaa at res.28 = (Tyr, Asn or Phe); Xaa at
res.31 = (Glu, His, Tyr, Asp, Gln or Ser); Xaa at
res.33 = Glu, Lys, Asp, Gln or Ala); Xaa at res.35 =
(Ala, Ser, Pro, Gln or Asn); Xaa at res.36 = (Phe, Leu
15 or Tyr); Xaa at res.38 = (Leu, Val or Met); Xaa at
res.39 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.40 =
(Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.41 = (Tyr,
Cys, His, Ser or Ile); Xaa at res.42 = (Met, Phe, Gly
or Leu); Xaa at res.43 = (Asn, Ser or Lys); Xaa at
20 res.44 = (Ala, Ser, Gly or Pro); Xaa at res.45 = (Thr,
Leu or Ser); Xaa at res.49 = (Ile, Val or Thr); Xaa at
res.50 = (Val, Leu or Ile); Xaa at res.51 = (Gln or
Arg); Xaa at res.52 = (Thr, Ala or Ser); Xaa at res.53
= (Leu or Ile); Xaa at res.54 = (Val or Met); Xaa at
25 res.55 = (His, Asn or Arg); Xaa at res.56 = (Phe, Leu,
Asn, Ser, Ala or Val); Xaa at res.57 = (Ile, Met, Asn,
Ala, Val or Leu); Xaa at res.58 = (Asn, Lys, Ala, Glu,
Gly or Phe); Xaa at res.59 = (Pro, Ser or Val); Xaa at
res.60 = (Glu, Asp, Gly, Val or Lys); Xaa at res.61 =
30 (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Ala, Pro or His);
Xaa at res.62 = (Val, Ala or Ile); Xaa at res.63 = (Pro
or Asp); Xaa at res.64 = (Lys, Leu or Glu); Xaa at
res.65 = (Pro or Ala); Xaa at res.68 = (Ala or Val);
Xaa at res.70 = (Thr, Ala or Glu); Xaa at res.71 =
35 (Gln, Lys, Arg or Glu); Xaa at res.72 = (Leu, Met or

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Val); Xaa at res.73 = (Asn, Ser, Asp or Gly); Xaa at
res.74 = (Ala, Pro or Ser); Xaa at res.75 = (Ile, Thr,
Val or Leu); Xaa at res.76 = (Ser, Ala or Pro); Xaa at
res.77 = (Val, Met or Ile); Xaa at res.79 = (Tyr or
5 Phe); Xaa at res.80 = (Phe, Tyr, Leu or His); Xaa at
res.81 = (Asp, Asn or Leu); Xaa at res.82 = (Asp, Glu,
Asn or Ser); Xaa at res.83 = (Ser, Gln, Asn, Tyr or
Asp); Xaa at res.84 = (Ser, Asn, Asp, Glu or Lys); Xaa
at res.85 = (Asn, Thr or Lys); Xaa at res.87 = (Ile,
10 Val or Asn); Xaa at res.89 = (Lys or Arg); Xaa at
res.90 = (Lys, Asn, Gln, His or Val); Xaa at res.91 =
(Tyr or His); Xaa at res.92 = (Arg, Gln, Glu or Pro);
Xaa at res.93 = (Asn, Glu or Asp); Xaa at res.95 =
(Val, Thr, Ala or Ile); Xaa at res.97 = (Arg, Lys, Val,
15 Asp or Glu); Xaa at res.98 = (Ala, Gly, Glu or Ser);
Xaa at res.100 = (Gly or Ala); and Xaa at res.102 =
(His or Arg).

Particularly useful sequences for use as morphogens in
20 this invention include the C-terminal domains, e.g., the C-
terminal 96-102 amino acid residues of Vgl, Vgr-1, DPP,
OP-1, OP-2, CBMP-2A, CBMP-2B, GDF-1 (see Table II, below,
and Seq. ID Nos. 5-14), as well as proteins comprising the
C-terminal domains of 60A, BMP3, BMP5 and BMP6 (see Seq. ID
25 Nos. 24-28), all of which include at least the conserved six
or seven cysteine skeleton. In addition, biosynthetic
constructs designed from the generic sequences, such as
COP-1, 3-5, 7, 16, disclosed in U.S. Pat. No. 5,011,691,
also are useful. Other sequences include the
30 inhibins/activin proteins (see, for example, U.S. Pat.
Nos. 4,968,590 and 5,011,691). Accordingly, other useful
sequences are those sharing at least 70% amino acid sequence
homology or "similarity", and preferably 80% homology or
similarity with any of the sequences above. These are
35 anticipated to include allelic, species variants and other

sequence variants (e.g., including "muteins" or "mutant proteins"), whether naturally-occurring or biosynthetically produced, as well as novel members of this morphogenic family of proteins. As used herein, "amino acid sequence
5 homology" is understood to mean amino acid sequence similarity, and homologous sequences share identical or similar amino acids, where similar amino acids are conserved amino acids as defined by Dayoff et al., Atlas of Protein Sequence and Structure; vol.5, Suppl.3, pp.345-362 (M.O.
10 Dayoff, ed., Nat'l BioMed. Research Fdn., Washington D.C. 1978.) Thus, a candidate sequence sharing 70% amino acid homology with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 70% of the amino acids in the candidate sequence
15 are identical to the corresponding amino acid in the reference sequence, or constitute a conserved amino acid change thereto. "Amino acid sequence identity" is understood to require identical amino acids between two aligned sequences. Thus, a candidate sequence sharing 60%
20 amino acid identity with a reference sequence requires that, following alignment of the candidate sequence with the reference sequence, 60% of the amino acids in the candidate sequence are identical to the corresponding amino acid in the reference sequence.

25

As used herein, all homologies and identities calculated use OP-1 as the reference sequence. Also as used herein, sequences are aligned for homology and identity calculations using the method of Needleman et al. (1970) J.Mol. Biol.
30 48:443-453 and identities calculated by the Align program (DNASTar, Inc.) In all cases, internal gaps and amino acid insertions in the candidate sequence as aligned are ignored when making the homology/identity calculation.

- 30 -

The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein. Accordingly, in another preferred aspect of the invention, useful morphogens include active proteins comprising species of polypeptide chains having the generic amino acid sequence herein referred to as "OPX" (Seq. ID No. 29), which defines the seven cysteine skeleton and accommodates the homologies between the various identified species of OP1 and OP2. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos. 16-23).

20

In still another preferred aspect of the invention, useful morphogens include dimeric proteins comprising amino acid sequences encoded by nucleic acids that hybridize to DNA or RNA sequences encoding the C-terminal sequences defining the conserved seven cysteine domain of OP1 or OP2, e.g., nucleotides 1036-1341 and nucleotides 1390-1695 of Seq. ID No. 16 and 20, respectively, under stringent hybridization conditions. As used herein, stringent hybridization conditions are defined as hybridization in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

25

30

The morphogens useful in the methods, compositions and devices of this invention include proteins comprising any of

35

the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by recombinant DNA or other synthetic techniques, and includes allelic and species variants of these proteins, naturally-occurring or biosynthetic mutants thereof, as well as various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including those which may alter the conserved C-terminal cysteine skeleton, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the specifically described constructs disclosed herein. The proteins may include forms having varying glycosylation patterns, varying N-termini, a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in procaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently preferred host cells include E. coli or mammalian cells, such as CHO, COS or BSC cells. A detailed description of the morphogens useful in the methods, compositions and devices of this invention is disclosed in copending US patent application Serial Nos. 752,764, filed August 30, 1991, and 667,724, filed March 11, 1991, the disclosures of which are incorporated herein by reference.

Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or genomic libraries of various different species which encode appropriate amino acid sequences, or construct DNAs from oligonucleotides, and

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then can express them in various types of host cells,
including both procaryotes and eucaryotes, to produce large
quantities of active proteins capable of stimulating the
morphogenesis of, and/or inhibiting damage to, periodontal
5 tissue.

Other features and advantages of the invention will be
apparent from the following description of the preferred
embodiments, and from the claims.

Brief Description of the Drawings

The foregoing and other objects and features of this invention, as well as the invention itself, may be more
5 fully understood from the following description, when read together with the accompanying drawings, in which:

Fig. 1 is a schematic illustration of a healthy tooth in the tooth socket; and

10

Fig. 2 (A and B) are photomicrographs demonstrating the effect of morphogen (2A) or carrier alone (2B) on periodontal tissue regeneration in a surgically prepared canine tooth socket.

15

Fig.3 (A and B) are photomicrographs demonstrating the effect of morphogen (3A) or carrier alone (3B) on dentine tissue regeneration in a surgically exposed dental pulp experiment.

Detailed Description

It has been discovered that the morphogens described herein can stimulate periodontal tissue formation, including
5 regenerating lost or damaged periodontal ligament and/or cementum. The invention may be used for tooth implant integration as well as to inhibit and/or repair periodontal tissue loss due to disease or mechanical injury. The invention is practiced using a morphogen or morphogen-
10 stimulating agent, as defined herein, and according to the procedures described herein.

Provided below is a description of tooth anatomy and useful morphogens, including methods for their production
15 and formulation, as well as exemplary, non-limiting examples which (1) demonstrate the suitability of the morphogens described herein in the methods of the invention, and (2) provide assays with which to test candidate morphogens for their efficacy.

20

I. Tooth Anatomy

A vertical section of a tooth in the tooth socket is shown schematically in Fig. 1. The crown 6 of the tooth is
25 composed of enamel 8 and dentin 22. The pulp chamber 12 is seen in the interior of the crown 6 and the center of the root 10; it extends downward into the bony area 14, 16, 18 and opens by a minute orifice, the apical foramen 20, at the extremity of the root 10. The pulp chamber 12 contains
30 dental pulp, a loose connective tissue richly supplied with vessels and nerves, which enter the cavity through the apical foramen 20. Some of the cells of the pulp, i.e., odontoblasts, the precursors of dentin 22, are arranged as a layer on the wall of the pulp chamber 12. During
35 development of the tooth, odontoblasts are columnar, but

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later, after the dentin 22 is fully formed, they become flattened and resemble osteoblasts.

The solid portion of the mature tooth includes dentin 5 22, enamel 8, and a thin layer of cementum 24, which is disposed on the surface of the root 25. Enamel 8 is formed during development of the tooth from amyloblasts, and cementum 24 is formed from cementoblasts. In a fully developed tooth, the principal mass of the tooth comprises 10 dentin 22, which is made up of hydroxyapatite crystals embedded in a strong meshwork of collagen fibers. The dentin includes a number of minute wavy and branching tubes called dental canaliculi, embedded in a dense homogeneous substance, the matrix. The dental canaliculi are parallel 15 with one another and open at their inner ends into the pulp chamber 12. The dentin matrix is translucent and comprises the majority of the inorganic mass of the dentin. It includes a number of fine fibrils, which are continuous with the fibrils of the dental pulp. After the organic matter 20 has been removed by steeping a tooth in weak acid, the remaining organic matter may be torn into laminae that run parallel with the pulp chamber 12 across the direction of the tubes.

25 The cementum 24 is disposed as a thin mineralized layer covering the tooth root. It extends from where the enamel terminates to the apex of each root, where it is usually very thick. Cementum resembles bone in structure and chemical composition in that it contains, sparingly, the 30 lacunae and canaliculi that characterize true bone; in the thicker portions of the cementum, the lamellae and Haversian canals peculiar to bone are also found. As a result of aging, the cementum increases in thickness and the pulp chamber also becomes partially filled with a hard substance 35 that is intermediate in structure between dentin and bone.

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It appears to be formed by a slow conversion of the dental pulp, which shrinks or even disappears.

The periodontal ligament, or periodontal membrane 26, is
5 the layer of periodontal tissue which forms a cushion
between the cementum 24 and the bone 14, 16, 18; it holds
the tooth in position by suspending it in the socket
(alveolus) of the jawbone. The periodontal ligament is a
highly organized tissue which is formed from periodontal
10 fibroblasts. It organizes the collagen fibers which pass
directly from the bone of the jaw into the cementum.

II. Useful Morphogens

15 As defined herein a protein is morphogenic if it is
capable of inducing the developmental cascade of cellular
and molecular events that culminate in the formation of new,
organ-specific tissue and comprises at least the conserved
C-terminal six cysteine skeleton or its functional
20 equivalent (see supra). Specifically, the morphogens
generally are capable of all of the following biological
functions in a morphogenically permissive environment:
stimulating proliferation of progenitor cells; stimulating
the differentiation of progenitor cells; stimulating the
25 proliferation of differentiated cells; and supporting the
growth and maintenance of differentiated cells. Details of
how the morphogens useful in the method of this invention
first were identified, as well as a description on how to
make, use and test them for morphogenic activity are
30 disclosed in international application (US92/01968
(W092/15323), the disclosure of which is incorporated
hereinabove by reference. As disclosed therein, the
morphogens may be purified from naturally-sourced material
or recombinantly produced from procaryotic or eucaryotic
35 host cells, using the genetic sequences disclosed therein.

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Alternatively, novel morphogenic sequences may be identified following the procedures disclosed therein.

Particularly useful proteins include those which
5 comprise the naturally derived sequences disclosed in Table II. Other useful sequences include biosynthetic constructs such as those disclosed in U.S. Pat. 5,011,691, the disclosure of which is incorporated herein by reference (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).
10

Accordingly, the morphogens useful in the methods and compositions of this invention also may be described by morphogenically active proteins having amino acid sequences sharing 70% or, preferably, 80% homology (similarity) with
15 any of the sequences described above, where "homology" is as defined herein above.

The morphogens useful in the method of this invention also can be described by any of the 6 generic sequences
20 described herein (Generic Sequences 1, 2, 3, 4, 5 and 6). Generic sequences 1 and 2 also may include, at their N-terminus, the sequence

Cys Xaa Xaa Xaa Xaa (Seq. ID No. 15)
25 1 5

Table II, set forth below, compares the amino acid sequences of the active regions of native proteins that have been identified as morphogens, including human OP-1 (hOP-1,
30 Seq. ID Nos. 5 and 16-17), mouse OP-1 (mOP-1, Seq. ID Nos. 6 and 18-19), human and mouse OP-2 (Seq. ID Nos. 7, 8, and 20-23), CBMP2A (Seq. ID No. 9), CBMP2B (Seq. ID No. 10), BMP3 (Seq. ID No. 26), DPP (from Drosophila, Seq. ID No. 11), Vgl, (from Xenopus, Seq. ID No. 12), Vgr-1 (from mouse, Seq.
35 ID No. 13), GDF-1 (from mouse, Seq. ID Nos. 14, 32 and 33),

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60A protein (from *Drosophila*, Seq. ID Nos. 24 and 25), BMP5 (Seq. ID No. 27) and BMP6 (Seq. ID No. 28). The sequences are aligned essentially following the method of Needleman et al. (1970) J. Mol. Biol., 48:443-453, calculated using the

5 Align Program (DNASTar, Inc.) In the table, three dots indicates that the amino acid in that position is the same as the amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino

10 acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile.

15

TABLE II

	hOP-1	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
20	mOP-1
	hOP-2	...	Arg	Arg
	mOP-2	...	Arg	Arg
	DPP	...	Arg	Arg	...	Ser
	Vgl	Lys	Arg	His
25	Vgr-1	Gly
	CBMP-2A	Arg	...	Pro
	CBMP-2B	...	Arg	Arg	...	Ser
	BMP3	...	Ala	Arg	Arg	Tyr	...	Lys	...
	GDF-1	...	Arg	Ala	Arg	Arg
30	60A	...	Gln	Met	Glu	Thr
	BMP5
	BMP6	...	Arg
		1				5			

35

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	hOP-1	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
	mOP-1
	hOP-2	Gln	Leu	...
	mOP-2	Ser	Leu	...
5	DPP	Asp	...	Ser	...	Val	Asp	...
	Vgl	Glu	...	Lys	...	Val	Asn
	Vgr-1	Gln	...	Val
	CBMP-2A	Asp	...	Ser	...	Val	Asn	...
	CBMP-2B	Asp	...	Ser	...	Val	Asn	...
10	BMP3	Asp	...	Ala	...	Ile	Ser	Glu
	GDF-1	Glu	Val	His	Arg
	60A	Asp	...	Lys	His	...
	BMP5
	BMP6	Gln
15			10					15		
	hOP-1	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
	mOP-1
	hOP-2	...	Val	Gln	Ser
20	mOP-2	...	Val	Gln	Ser
	DPP	Val	Leu	Asp
	Vgl	...	Val	Gln	Met
	Vgr-1	Lys
	CBMP-2A	Val	Pro	His
25	CBMP-2B	Val	Pro	Gln
	BMP3	Ser	...	Lys	Ser	Phe	Asp
	GDF-1	...	Val	Arg	...	Phe	Leu
	60A	Gly
	BMP5
30	BMP6	Lys
				20					25	

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	hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
	mOP-1
	hOP-2	Ser
	mOP-2
5	DPP	His	...	Lys	...	Pro
	Vgl	...	Asn	Tyr	Pro
	Vgr-1	...	Asn	Asp	Ser
	CBMP-2A	...	Phe	His	...	Glu	...	Pro
	CBMP-2B	...	Phe	His	...	Asp	...	Pro
10	BMP3	Ser	...	Ala	...	Gln
	GDF-1	...	Asn	Gln	...	Gln
	60A	...	Phe	Ser	Asn
	BMP5	...	Phe	Asp	Ser
	BMP6	...	Asn	Asp	Ser
15					30					35
	hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
	mOP-1
	hOP-2	Asp	...	Cys
20	mOP-2	Asp	...	Cys
	DPP	Ala	Asp	His	Phe	...	Ser
	Vgl	Tyr	Thr	Glu	Ile	Leu	...	Gly
	Vgr-1	Ala	His
	CBMP-2A	Ala	Asp	His	Leu	...	Ser
25	CBMP-2B	Ala	Asp	His	Leu	...	Ser
	GDF-1	Leu	...	Val	Ala	Leu	Ser	Gly	Ser**	...
	BMP3	Met	Pro	Lys	Ser	Leu	Lys	Pro
	60A	Ala	His
	BMP5	Ala	His	Met
30	BMP6	Ala	His	Met
					40					

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	hOP-1	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
	mOP-1
	hOP-2	Leu	...	Ser	...
	mOP-2	Leu	...	Ser	...
5	DPP	Val
	Vgl	Ser	Leu
	Vgr-1
	CBMP-2A
	CBMP-2B
10	BMP3	Ser	Thr	Ile	...	Ser	Ile
	GDF-1	Leu	Val	Leu	Arg	Ala	...
	60A
	BMP5
	BMP6
15		45					50			
	hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
	mOP-1	Asp
20	hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
	mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
	DPP	...	Asn	Asn	Asn	Gly	Lys	...
	Vgl	Ser	...	Glu	Asp	Ile
	Vgr-1	Val	Met	Tyr	...
25	CBMP-2A	...	Asn	Ser	Val	...	Ser	---	Lys	Ile
	CBMP-2B	...	Asn	Ser	Val	...	Ser	---	Ser	Ile
	BMP3	...	Arg	Ala**	Gly	Val	Val	Pro	Gly	Ile
	GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
	60A	Leu	Leu	Glu	...	Lys	Lys	...
30	BMP5	Leu	Met	Phe	...	Asp	His	...
	BMP6	Leu	Met	Tyr	...
			55					60		

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	hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
	mOP-1
	hOP-2	Ala	Lys
	mOP-2	Ala	Lys
5	DPP	Ala	Val
	Vgl	...	Leu	Val	Lys
	Vgr-1	Lys
	CBMP-2A	Ala	Val	Glu
	CBMP-2B	Ala	Val	Glu
10	BMP3	...	Glu	Val	...	Glu	Lys
	GDF-1	Asp	Leu	Val	...	Ala	Arg
	60A	Arg
	BMP5	Lys
	BMP6	Lys
15				65					70	
	hOP-1	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	mOP-1
	hOP-2	...	Ser	...	Thr	Tyr
20	mOP-2	...	Ser	...	Thr	Tyr
	Vgl	Met	Ser	Pro	Met	...	Phe	Tyr
	Vgr-1	Val
	DPP	...	Asp	Ser	Val	Ala	Met	Leu
	CBMP-2A	...	Ser	Met	Leu
25	CBMP-2B	...	Ser	Met	Leu
	BMP3	Met	Ser	Ser	Leu	...	Ile	...	Phe	Tyr
	GDF-1	...	Ser	Pro	Phe	...
	60A	...	Gly	...	Leu	Pro	His
	BMP5
30	BMP6
				75					80	

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	hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
	mOP-1
	hOP-2	...	Ser	...	Asn	Arg
	mOP-2	...	Ser	...	Asn	Arg
5	DPP	Asn	...	Gln	...	Thr	...	Val
	Vgl	...	Asn	Asn	Asp	Val	...	Arg
	Vgr-1	Asn
	CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val
	CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val
10	BMP3	...	Glu	Asn	Lys	Val
	GDF-1	...	Asn	...	Asp	Val	...	Arg
	60A	Leu	Asn	Asp	Glu	Asn
	BMP5
	BMP6	Asn
15						85				
	hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg	
	mOP-1	
20	hOP-2	...	His	Lys	
	mOP-2	...	His	Lys	
	DPP	Asn	...	Gln	Glu	...	Thr	...	Val	
	Vgl	His	...	Glu	Ala	...	Asp	
	Vgr-1	
25	CBMP-2A	Asn	...	Gln	Asp	Glu	
	CBMP-2B	Asn	...	Gln	Glu	Glu	
	BMP3	Val	...	Pro	Thr	...	Glu	
	GDF-1	Gln	...	Glu	Asp	Asp	
	60A	Ile	...	Lys	
30	BMP5	
	BMP6	Trp	
		90					95			

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	hOP-1	Ala	Cys	Gly	Cys	His
	mOP-1
	hOP-2
	mOP-2
5	DPP	Gly	Arg
	Vgl	Glu	Arg
	Vgr-1
	CBMP-2A	Gly	Arg
	CBMP-2B	Gly	Arg
10	BMP3	Ser	...	Ala	...	Arg
	GDF-1	Glu	Arg
	60A	Ser
	BMP5	Ser
	BMP6
15				100		

**Between residues 56 and 57 of BMP3 is a Val residue;
 between residues 43 and 44 of GDF-1 lies
 the amino acid sequence Gly-Gly-Pro-Pro.

20

As is apparent from the foregoing amino acid sequence comparisons, significant amino acid changes can be made within the generic sequences while retaining the morphogenic activity. For example, while the GDF-1 protein sequence depicted in Table II shares only about 50% amino acid identity with the hOP1 sequence described therein, the GDF-1 sequence shares greater than 70% amino acid sequence homology (or "similarity") with the hOP1 sequence, where "homology" or "similarity" includes allowed conservative amino acid changes within the sequence as defined by Dayoff, et al., Atlas of Protein Sequence and Structure vol.5, supp.3, pp.345-362, (M.O. Dayoff, ed., Nat'l BioMed. Res. Fd'n, Washington D.C. 1979.)

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The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six
5 cysteine skeleton of hOP1 (e.g., residues 43-139 of Seq. ID No. 5). These most preferred sequences include both allelic and species variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in still another preferred aspect, the invention includes morphogens
10 comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine skeleton and accommodates the identities between the various identified mouse and human OP1 and OP2 proteins. OPX is presented in Seq. ID No. 29.
15 As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human OP1 or OP2 (see Seq. ID Nos. 5-8 and/or Seq. ID Nos. 16-23).

20

Alternatively, an effective amount of an agent capable of stimulating endogenous morphogen levels may be administered by any of the routes described herein below. For example, an agent capable of stimulating morphogen
25 production and/or secretion from periodontal tissue cells, alveolar bone tissue cells in the fresh tooth socket, or dentin tissue, may be provided to a mammal, e.g., by direct administration of the morphogen-stimulating agent to the tooth root and/or tooth socket bone surface. Alternatively,
30 the morphogen-stimulating agent may induce morphogen expression and/or secretion at a distant site (e.g., at a tissue locus other than periodontal, dental or alveolar bone tissue), with the expressed morphogen targeting itself to periodontal tissue. A method for identifying and testing
35 agents capable of modulating the levels of endogenous

morphogens in a given tissue is described generally herein in Example 3, and in detail in copending USSN [Atty Docket CRP-058CP], filed August 28, 1992 and USSN 752,859, filed August 30, 1991, the disclosures of which are incorporated
5 herein by reference. Briefly, candidate compounds can be identified and tested by incubating the compound in vitro with a test tissue or cells thereof, for a time sufficient to allow the compound to affect the production, i.e., the expression and/or secretion, of a morphogen produced by the
10 cells of that tissue. Here, suitable tissue, or cultured cells of a tissue, preferably comprise periodontal fibroblasts, cementoblasts, odontoblasts or osteoblasts.

15 III. Formulations and Methods for Administration

1. Therapeutic Agent Considerations

The morphogens may be provided to the tooth root and/or tooth socket surface by any suitable means. Preferably, the
20 morphogen, or a morphogen-stimulating agent, (collectively, the therapeutic agent) is provided directly to the tissue surface by topical administration. Alternatively, the therapeutic agent may be provided to the tissue by, for example, local injection. While not currently preferred,
25 systemic injection also may be a viable administration route for certain applications, such as periodontal tissue maintenance in older adults, immuno-suppressed individuals, or others at chronic risk for periodontal tissue loss. A detailed description of considerations for systemic
30 administration, including oral and parenteral administration, is disclosed, for example, in international application US92/07358 (WO93/04692), incorporated hereinabove by reference.

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Where the therapeutic agent is provided directly to the tooth socket, the therapeutic agent may be provided to the socket surface as part of a biocompatible formulation that may be a liquid, gel or solid. The therapeutic agent
5 further may be dispersed in and associated with a carrier capable of maintaining the morphogen at the administered locus. Useful formulations include viscous compositions. Biocompatible compositions that increase the viscosity of the formulation include glycerol, polyalkylene glycols such
10 as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like.

The formulation also may include an in vivo bioresorbable carrier material that acts as a controlled
15 release delivery vehicle. Useful carriers may include biocompatible, preferably biodegradable structural components from, e.g., an extracellular matrix, such as collagen, laminin, hyaluronic acid, and the like, or polymeric materials, such as polylactic, polybutyric and
20 polyglycolic acids. The carrier also may comprise an acellular tissue matrix, substantially depleted in nonstructural components, such as a demineralized, guanidine-extracted dentin, periodontal ligament or cementum matrix. Details for preparing such matrices are disclosed
25 in international application US92/01968 (W093/15323). Other useful controlled release carriers in which the therapeutic agent may be dispersed are described in U.S. Pat. Nos. 4,975,526 and 4,919,939, the disclosures of which are incorporated herein by reference.

30

Where the morphogen is to be provided to a tooth root surface, it may be formulated in a composition for controlled delivery as described above and applied topically to the tooth root surface as described below.

35 Alternatively, or in addition, the therapeutic agent may be

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dispersed in a liquid formulation into which at least the tooth root surface is placed and the liquid lyophilized to adsorb the therapeutic agent onto the tooth surface.

5 Where the agent is administered to inhibit periodontal tissue loss and/or to regenerate periodontal tissue surrounding an implanted tooth, the agent may be provided to the area between the tooth and gum (gingiva) by injection or by topical application.

10

Where the morphogen is to be provided directly (e.g., locally, as by injection, e.g., to a periodontal or alveolar tissue site), the morphogen preferably comprises part of an aqueous solution which also may contain a carrier material.

15 The solution is physiologically acceptable so that in addition to delivery of the desired morphogen to the patient, the solution does not otherwise adversely affect the patient's electrolyte and volume balance. The aqueous medium for the morphogen thus may comprise normal
20 physiologic saline (0.85% NaCl, 0.15M), pH 7-7.4. The aqueous solution containing the morphogen can be made, for example, by dissolving the protein in 50% ethanol containing acetonitrile in 0.1% trifluoroacetic acid (TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant
25 solution then is added, for example, to ten volumes of phosphate buffered saline (PBS), which further may include 0.1-0.2% human serum albumin (HSA). The resultant solution preferably is vortexed extensively. If desired, a given morphogen may be made more soluble in the solution by
30 association with a suitable molecule. For example, the pro form of the morphogenic protein comprises a species that is soluble in physiological solutions. In fact, the endogenous protein is thought to be transported (e.g., secreted and circulated) in this form. This soluble form of the protein
35 may be obtained from the culture medium of

morphogen-secreting mammalian cells. Alternatively, a soluble species may be formulated by complexing the mature dimer (or an active fragment thereof) with part or all of a pro domain. Other components, including various serum
5 proteins, also may be useful. A more detailed description for formulating soluble morphogen complexes appears in Example 4, below.

Finally, the morphogens or morphogen-stimulating agents
10 provided herein may be administered alone or in combination with other molecules, particularly symptom alleviating cofactors. Useful pharmaceutical cofactors include antiseptics, antibiotics, anaesthetics and analgesics. Preferred antiseptics for use in the present system include
15 chlorhexidine and tibezoneium iodide; preferred antibiotics include tetracycline, aminoglycosides such as neomycin, gentamycin, kanamycin, tobramycin, netilmicin, sisomicin, amikamycin, their sulfates or other derivatives, macrolides such as erythromycin, its salts and other derivatives,
20 spiramycin, josamicin or miocamicin, penicillins such as ampicillin, amoxicillin and the like, and cephalosporins, for example, cefaclor, cefadroxil, cefazolin, cefoperazone, cefotaxime, cephalothin, cefalexin, ceforanide, cefonicide or ceftriaxone. Preferred anaesthetics/analgesics include
25 amide-type local anaesthetics such as lidocaine, mepivacaine, pyrrocaine, bupivacaine, prilocaine, etidocaine, or other widely used anaesthetics such as procaine.

30 Other cofactors include non-steroidal anti-inflammatory agents. However, the morphogens described herein themselves modulate the body's inflammatory/immune response to an initial tissue injury. Specifically, and as described in detail in international application US92/07358 (WO93/04692),
35 in the presence of a morphogen, progenitor inflammatory

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effector cells induced to migrate to a site of tissue injury do not become significantly activated. Without being limited to any given theory, it is thought that, in the presence of the morphogen, damaged tissue is induced to
5 undergo a recapitulation of tissue morphogenesis, where progenitor cells are induced to proliferate and differentiate in a tissue-specific manner, and new, functional, organized tissue is formed to replace the damaged or lost tissue, rather than disorganized, fibrous
10 scar tissue.

The formulated compositions contain therapeutically effective amounts of the morphogen, e.g., amounts which provide appropriate concentrations of the morphogen to the
15 tooth surface for a time sufficient to stimulate growth and development of periodontal tissues, including morphogenesis of periodontal ligament and/or cementum, and/or to substantially inhibit periodontal tissue loss.

20 As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition will vary depending upon a number of factors, including the biological efficacy of the selected morphogen, the chemical characteristics (e.g., hydrophobicity) of the
25 compounds employed, the formulation of the compound excipients, the administration route, and the treatment envisioned. The preferred dosage to be administered also is likely to depend on such variables such as the condition of the tissues within the tooth socket, the size of the tooth
30 or tooth socket, the length of time after tooth loss, extent of periodontal tissue loss and the overall health status of the particular patient. The amount of morphogen applied also will depend on the tooth size. In general, 0.1-1000 μg of morphogen are sufficient with 1-100 μg being preferable.
35 For example, for a large tooth, e.g., an incisor or large

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molar, about 10-100 μg , and preferably 50 μg of morphogen, may be used to advantage; a medium tooth may be treated with approximately 5-50 μg , and preferably 25 μg ; and a small tooth, with approximately 1-25, preferably 5-10 μg morphogen. No obvious morphogen induced pathological lesions are induced when mature morphogen (e.g., OP-1, 20 μg) is administered daily to normal growing rats for 21 consecutive days. Moreover, 10 μg systemic injections of morphogen (e.g., OP-1) injected daily for 10 days into normal newborn mice does not produce any gross abnormalities.

2. Tooth Preparation

15 Tooth loss may be repaired by implanting a viable tooth having a healthy root and pulp system or by implanting a tooth prosthesis. The prosthesis may be a tooth from which the root has been removed and replaced with a biocompatible, biologically inert material, e.g., as typically is replaced
20 in a root canal procedure, or may be a completely synthetic prosthesis coated, for example, with a porous material to enhance tooth integration in the tooth socket. Useful prosthesis coating materials include collagen fibers, ceramics and metals, such as titanium oxide. The root of
25 the implanted tooth first may be partially demineralized as described below. Alternatively, a clean, mineralized natural tooth or dentin-containing prosthetic tooth may be implanted.

30 A tooth to be implanted first is obtained, e.g., by loss or removal of a natural tooth from the tooth socket, e.g., using standard tooth extraction means well known to one skilled in the dentistry art. Alternatively, an allogenic tooth may be obtained from a tooth bank. The natural,
35 mineralized tooth or tooth root may be coated as is with a

morphogen and implanted as described below. Alternatively, the mineralized, natural tooth root surface first may be scored or scraped to expose dentin tissue beneath the enamel. Natural, mineralized teeth also may be treated

5 briefly with an acidic solution (e.g., sodium citrate, about pH 3.5) to remove a thin external layer, e.g., about 1-5 cells in thickness from at least the root surface. Preferred treatment times are from about 0.5 to 5 minutes. The treated teeth preferably then are washed, dried and

10 coated with morphogen as described below. Alternatively, the tooth root portion may be at least partially demineralized according to any conventional procedure prior to implantation. A currently preferred demineralization method is to soak the tooth in a demineralizing solution for

15 a length of time sufficient to remove at least some mineral components from the tooth. For example, at least the root portion of the tooth may be placed in a volume, e.g., 0.025-1 liter of a demineralizing agent such as hydrochloric acid (HCl) at a cool temperature for a time sufficient to achieve

20 partial demineralization, e.g., 0.5-0.6 M HCl at 4°C for a prescribed number of minutes (e.g., preferably within the range of about 10-200 minutes.) Essentially complete demineralization may be achieved by acid exposure for 1-7 days. If desired, several changes of the demineralizing

25 agent may be performed. The partially demineralized tooth will be of the same shape as prior to demineralization, but will weigh less due to the absence of the mineral content. The tooth then may be dried by lyophilization.

30 The tooth or tooth prosthesis may be treated with morphogenic protein as follows. The morphogen may be applied to the tooth or tooth prosthesis root surface by any means known in the art for adsorbing a protein to a surface. A currently preferred method is to suspend the morphogen in

35 a small volume sufficient to cover the tooth surface, e.g.,

200-300 μ l, freeze the tooth in solution, and then lyophilize the frozen liquid. A currently preferred solution is ethanol (e.g. 50%) or acetonitrile/trifluoroacetic acid (TFA), other solutions include HCL/TFA, buffered saline, and the
5 like. Alternatively, or in addition, the therapeutic agent may be provided to the tooth root surface dispersed in a suitable carrier material as described above. Similarly, and as described above, the therapeutic agent may be provided to the tooth socket surface and the tooth to be
10 implanted embedded in the morphogen composition on the socket surface. Also as described above, the morphogen may be provided to the tooth root surface in admixture with one or more cofactors.

15 The tooth then is implanted into a fresh or surgically prepared tooth socket. A surgically prepared surface is prepared by extracting the tooth and removing any scar or other undesired fibrous tissue built up in the socket by standard mechanical and/or chemical procedures well known on
20 the surgical and dental arts. The tooth then is implanted in the site using standard dental and surgical procedures.

The implanted tooth is allowed to grow in the prepared socket for a time sufficient to allow the periodontium to
25 regenerate, e.g., one to several months. The integrity and health of the integrated tooth then may be assessed by a dentist by radiography and visual examination.

For experimental purposes, the integration of an
30 implanted tooth following morphogen treatment can be assessed for integrity and health by removing the entire mandibular area, including the tooth socket and tooth, and examining cross sections of the mandibular area. 5-10 μ m cross sections may be prepared for histological evaluation
35 by standard histology procedures, e.g., fixing tissue with

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formalin, preparing sections for slides and staining with eosin and hematoxylin. The growth and integrity of hard tissues, such as bone, cementum and dentin, also can be evaluated radiographically.

5

Finally, as described in Example 2 below, the morphogens of this invention also induce dentin tissue morphogenesis when provided to an area of lost or damaged dentin. Accordingly, using the procedures described herein and in international application (US92/01968 (WO92/15323)), the morphogen described herein also may be used to repair and regenerate damaged and/or lost dentin tissue in an implanted tooth.

15

IV. Examples

Example 1. Experimental Regeneration of Peridontium in a Dog Model

20

The following experiment demonstrates successful integration of an implanted demineralized, protein-extracted morphogen-treated tooth in a mammal. Premolar teeth were extracted from a dog and divided into three experimental groups: (a) demineralized teeth; (b) demineralized and guanidine extracted teeth; and (c) demineralized, guanidine extracted, and morphogen-treated teeth. Teeth from each group were tested in "fresh" sockets, e.g., tooth sockets from which the teeth had just been removed, as well as surgically prepared sockets, e.g., sockets from which teeth had been extracted 2 months previously and in which scar tissue had formed. These "healed" sockets were surgically prepared for tooth implantation by removing (e.g., by scraping) scar tissue build up to reveal fresh alveolar bone.

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The teeth from all three groups were completely demineralized by placing them in 4 liters of 0.5 M HCl at 4°C for 5 days. The 0.5 M HCl solution was changed every 24 hours during the 5 day period. The teeth then were washed
5 in 4 liters of deionized water at 4°C for 5 days. The water solution was also changed every 24 hours during the 5 day period. Teeth from group (a) then were lyophilized until dry and set aside and maintained at 4°C until ready for use.

10 Teeth from groups (b) and (c) then were protein-extracted by multiple extractions in 6 M guanidine HCl, followed by washes with distilled water. Specifically, the teeth were placed in in 2-4 liters of 6 M guanidine-HCl/Tris HCl pH 7.0 at 4°C for 72 hours; then washed and
15 further extracted in 200 ml of the guanidine-HCl solution for 4 hours. The teeth were washed again with 4 liters of distilled dH₂O at 4°C for 48 hours, and 4 liters of dH₂O for an additional 12 hours with 3 changes of dH₂O. The teeth were then lyophilized until dry. Teeth from group (b) were
20 then set aside and maintained at 4°C until ready for use.

Teeth from group (c) then were treated with the morphogen OP-1 as follows. 1.15 mg of OP-1 was resuspended in 4 ml of 47.5% ethanol/0.09% trifluoroacetic acid (TFA).
25 The concentration was determined to be 0.273 mg/ml. Approximately 50 µg of OP-1 (183 µl of the OP-1 solution) was dispensed into an eppendorf tube, and the total volume brought to 300 µl of 47.5% ethanol/0.09% TFA. Each tooth then was placed in an eppendorf tube such that the OP-1
30 solution just covered the tooth. The tube was placed at -70°C until the OP-1 solution was frozen, and lyophilized until dry. During lyophilization, care was taken to keep the tube cold. Approximately 50-70% of the OP-1 can be expected to remain in or on the tooth after lyophilization.

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The teeth from each of groups (a), (b), and (c) were then implanted into a freshly prepared tooth socket or surgically prepared socket using standard dental surgery procedures known in the art.

5

The implanted teeth in all three groups were allowed to remain in the socket for two months. The dog then was sacrificed, the mandible cross-sectioned and x-rayed, and histology performed. The results are described below and
10 follows.

Ankylosis formed in the group (a) implants, where demineralized tooth matrix was implanted alone. Cross-sections of the group (a) mandible revealed that the
15 demineralized tooth was surrounded by bone directly attaching to the root or dentin surface. In addition, there was little new tissue growth between the tooth and the bone. Representative histology is illustrated in the photomicrograph of Fig. 2A where bone tissue 14 grows
20 directly into dental tissue 22 in the implanted tooth.

In the group (b) implants, cross-sections revealed formation of unorganized fibrous tissue around the implanted demineralized, guanidine extracted tooth. The periodontal
25 ligament was loose and disorganized, as was the surrounding bony tissue. Examination of the tooth root surface where cementum matrix normally appears revealed resorption of cementum in the upper coronal surface of the tooth. Histological sections also revealed inflammation as
30 evidenced by the presence of macrophages.

As is evident in Fig. 2b, group (c) implant cross-sections revealed formation of newly formed, organized cementum 24 and periodontal ligament tissue 26 around the
35 morphogen-treated tooth matrix, and growth of new bone

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connecting the newly formed periodontium to the mandible. The tooth was firmly anchored in the tooth socket. The tissues surrounding the tooth, i.e., the newly-formed cementum growing perpendicular to the newly-formed
5 periodontal ligament, and the alveolar bony tissue, all were healthy and organized much as the tooth and tooth socket shown schematically in Fig. 1. The newly-formed cementum comprised immature columnar cell layers which were beginning to flatten into mature cementoblasts, and the newly-formed
10 periodontal ligament comprised a thick layer of tissue to anchor and cushion the tooth within the tooth socket.

The results of this experiment demonstrate that morphogens promote tooth integration into a tooth socket,
15 and induce morphogenesis of periodontium, including morphogenesis of the regeneration and formation of the periodontium, new cementum and periodontal ligament.

Without being limited to any particular theory, the
20 morphogens may act in the tooth socket environment by inducing a differentiation of primary fibroblasts on the alveolar surface to differentiate into cementoblasts which then induct other primary fibroblasts to form periodontal ligament.

25

Example 2. Morphogen-Induced Dentinogenesis

The examples presented below demonstrate the efficacy of morphogens in inducing dentin tissue morphogenesis in an
30 animal model. Further details of the first experiment and the implications of this biological activity of morphogens are disclosed in international application (US92/01968 (WO92/15323)).

To date, the unpredictable response of dental pulp tissue to injury is a basic clinical problem in dentistry. Cynomolgus monkeys were chosen as primate models for the reparative dentine/pulp capping examples described below.

5

Using standard dental surgical procedures, small areas (e.g., 2mm) of dental pulps were surgically exposed by removing the enamel and dentin immediately above the pulp (by drilling) of sample teeth, performing a partial
10 amputation of the coronal pulp tissue, inducing hemostasis, application of the pulp treatment, and sealing and filling the cavity by standard procedures.

Pulp treatments used were: OP1 dispersed in a carrier
15 matrix; carrier matrix alone and no treatment. Twelve teeth per animal (four for each treatment) were prepared, and two animals were used. At four weeks, teeth were extracted and processed histologically for analysis of dentin formation, and/or ground to analyze dentin mineralization. Morphogen
20 treatment produced dramatic effects: Control treatments with carrier alone or with no treatment (PBS) showed little or no reparation of the lost tissue. By contrast, morphogen-treated teeth showed significant dentin tissue formation in the area where dentin tissue had been
25 surgically removed. The experimental results show that morphogen treatment reliably induced formation of reparative or osteodentin bridges on surgically exposed healthy dental pulps. See, for example, Fig. 3A, where OP1 dispersed in a carrier (demineralized, guanidine-extracted bone collagen
30 matrix prepared as described in U.S. Patent No. 4,975,526) constituted the pulp treatment. As is evident from the micrograph new dentine formation effectively bridges or "caps" the surgically exposed dental pulp, maintaining the integrity and viability of the pulp tissue. By contrast,
35 pulps treated with carrier matrix alone, or not treated,

failed to form reparative dentin. See, for example Fig. 3B where carrier alone (demineralized, guanidine-extracted bone collagen matrix prepared as described in U.S. Patent No. 4,975,526) constituted the pulp treatment. As is evident from the micrograph, minimal reparative dentin formed, insufficient to bridge the exposed pulp tissue. Without further treatment such exposed, unprotected pulp tissue will become infected and die.

- 10 In a supplemental experiment, a range of morphogen concentrations were tested. In all cases, human OP-1, prepared as described in Sampath et al. (1992) J. Biol. Chem. 267: 20352-20362, was the morphogen tested, and bone collagen matrix, prepared as described in U.S. Patent No. 4,975,526 was the carrier material/delivery vehicle ("CM"). Briefly, cortical bone powder was prepared from freshly obtained bovine femurs. The epiphyses, adherent flesh and marrow were removed and residual lipids extracted with hexane, isopropanol, and ethyl ether. The resulting material was ground and sieved to a described particle size of 75-425 μm . The cortical bone powder then was demineralized in acid, and soluble proteins extracted with guanidine hydrochloride. The demineralized, extracted bone powder then was subjected to a thermal acid treatment, washed with water, and lyophilized. The final dry powder was sieved to remove particles $>425 \mu\text{m}$ and stored at 4°C .

The hOP-1/CM samples were prepared by combining hOP-1 with the CM and drying under vacuum. The batch used in these experiments contained $2.5\mu\text{g}$ hOP-1/mg CM. Prior to implant the sample was moistened with a sterile aqueous solution, preferably saline, to form a paste-like substance. CM controls were prepared using the same procedure, omitting the morphogen. The samples were stored at -20°C until used.

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The pulp capping experiments were conducted using 4 adult female non-human primates (*Macaca fascicularis*) of approximately 4 kg each. The animals were sedated using standard procedures, e.g., with ketamine (15 mg/kg body wt.) and acepromazine (0.55 mg/kg body wt.) supplemented with local intraoral infiltration anesthesia.

Thirty premolar and molar teeth in four animals were isolated by rubber dam and the pulps exposed using standard dentistry procedures, e.g., using sterile high speed rotary cutting instruments with water spray coolant. The pulp exposures made were approximately 1-1.5 by 2-2.5 mm. Partial hemostasis was achieved with sterile cotton pellets but the teeth were not dried extensively prior to treatment. The exposed pulps were treated with: hOP-1/CM (2.5 μ g hOP-1/mg CM) at 1.5, 3.0 or 6.0 mg/tooth; or one of three controls: $\text{Ca}(\text{OH})_2$ paste, a standard pulp capping agent used in the art ("Dycal", L.D. Caulk, Milford, DE); CM alone, 3.0 mg/tooth; or no treatment material. The teeth then were sealed with a standard adhesive, e.g., Temp-Bond NETM (Kerr U.S.A., Romulus, MI). The teeth were allowed to heal for six weeks. No changes in behavior were noted by any of the animals during the healing period.

The animals were sacrificed six weeks following surgery and prepared for histomorphometric analysis using standard procedures. For example, teeth were fixed by immersion in 10% formalin in phosphate buffered saline (pH 7.2) and decalcified in formic acid/sodium citrate at room temperature for 6-8 days. The specimens were processed, imbedded in paraffin, serial sectioned (5 μ m) and stained.

In all teeth treated with hOP-1/CM and for all OP1 concentrations tested, reparative dentine sufficient to bridge the surgically created gap that exposed the

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underlying pulp tissue was formed. As in the previous experiment, the morphogen/CM device was resorbed in the healed teeth, and replaced with reparative dentine, fully integrated with the cut dentine at the exposure site. Also
5 as in the previous experiment, the pulp tissue beneath the cap appeared normal, with intact odontoblasts lining the pulp chamber. The amount of new dentine tissue increased as the amount of OP-1 provided in a sample was increased, indicating that the amount of reparative dentine formed was
10 related to the mass of OP1/CM administered. Pulp treatments using CM alone or no treatment did not succeed in bridging the exposure site and in several cases resulted in necrotic pulp tissue. Treatments using $\text{Ca}(\text{OH})_2$ succeeded in bridging the gap, but the paste remained and the bridge created lies
15 within the pulp chamber itself.

Example 3. Screening Assay for Candidate Compounds which
Alter Endogenous Morphogen Levels

20 Candidate compound(s) which may be administered to affect the level of a given morphogen may be found using the following screening assay, in which the level of morphogen production by a cell type which produces measurable levels of the morphogen is determined with and without incubating
25 the cell in culture with the compound, in order to assess the effects of the compound on the cell. This can be accomplished by detection of the morphogen either at the protein or RNA level. A more detailed description also may be found in international application US92/07359
30 (WO93/05172), incorporated hereinabove by reference.

3.1 Growth of Cells in Culture

Cell cultures of kidney, adrenals, urinary bladder,
35 brain, or other organs, may be prepared as described widely

in the literature. For example, kidneys may be explanted from neonatal or new born or young or adult rodents (mouse or rat) and used in organ culture as whole or sliced (1-4 mm) tissues. Primary tissue cultures and established cell lines, also derived from kidney, adrenals, urinary, bladder, brain, mammary, or other tissues may be established in multiwell plates (6 well or 24 well) according to conventional cell culture techniques, and are cultured in the absence or presence of serum for a period of time (1-7 days). Cells may be cultured, for example, in Dulbecco's Modified Eagle medium (Gibco, Long Island, NY) containing serum (e.g., fetal calf serum at 1%-10%, Gibco) or in serum-deprived medium, as desired, or in defined medium (e.g., containing insulin, transferrin, glucose, albumin, or other growth factors).

Samples for testing the level of morphogen production includes culture supernatants or cell lysates, collected periodically and evaluated for OP-1 production by immunoblot analysis (Sambrook et al., eds., 1989, Molecular Cloning, Cold Spring Harbor Press, Cold Spring Harbor, NY), or a portion of the cell culture itself, collected periodically and used to prepare polyA+ RNA for RNA analysis. To monitor de novo OP-1 synthesis, some cultures are labeled according to conventional procedures with an ³⁵S-methionine/³⁵S-cysteine mixture for 6-24 hours and then evaluated for OP-1 synthesis by conventional immunoprecipitation methods.

3.2 Determination of Level of Morphogenic Protein

In order to quantitate the production of a morphogenic protein by a cell type, an immunoassay may be performed to detect the morphogen using a polyclonal or monoclonal antibody specific for that protein. For example, OP-1 may

be detected using a polyclonal antibody specific for OP-1 in an ELISA, as follows.

1 μ g/100 μ l of affinity-purified polyclonal rabbit IgG
5 specific for OP-1 is added to each well of a 96-well plate and incubated at 37°C for an hour. The wells are washed four times with 0.167M sodium borate buffer with 0.15 M NaCl (BSB), pH 8.2, containing 0.1% Tween 20. To minimize
10 non-specific binding, the wells are blocked by filling completely with 1% bovine serum albumin (BSA) in BSB and incubating for 1 hour at 37°C. The wells are then washed four times with BSB containing 0.1% Tween 20. A 100 μ l aliquot of an appropriate dilution of each of the test
15 samples of cell culture supernatant is added to each well in triplicate and incubated at 37°C for 30 min. After incubation, 100 μ l biotinylated rabbit anti-OP-1 serum (stock solution is about 1 mg/ml and diluted 1:400 in BSB containing 1% BSA before use) is added to each well and
20 incubated at 37°C for 30 min. The wells are then washed four times with BSB containing 0.1% Tween 20. 100 μ l streptavidin-alkaline (Southern Biotechnology Associates, Inc. Birmingham, Alabama, diluted 1:2000 in BSB containing 0.1% Tween 20 before use) is added to each well and
25 incubated at 37°C for 30 min. The plates are washed four times with 0.5M Tris buffered Saline (TBS), pH 7.2. 50 μ l substrate (ELISA Amplification System Kit, Life Technologies, Inc., Bethesda, MD) is added to each well
30 incubated at room temperature for 15 min. Then, 50 μ l amplifier (from the same amplification system kit) is added and incubated for another 15 min at room temperature. The reaction is stopped by the addition of 50 μ l 0.3 M sulphuric acid. The OD at 490 nm of the solution in each well is recorded. To quantitate OP-1 in culture media, a OP-1 standard curve is performed in parallel with the test
35 samples.

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Polyclonal antibody may be prepared as follows. Each rabbit is given a primary immunization of 100 ug/500 μ l E. coli produced OP-1 monomer (amino acids 328-431 in SEQ ID NO:5) in 0.1% SDS mixed with 500 μ l Complete Freund's Adjuvant. The antigen is injected subcutaneously at multiple sites on the back and flanks of the animal. The rabbit is boosted after a month in the same manner using incomplete Freund's Adjuvant. Test bleeds are taken from the ear vein seven days later. Additional boosts and test bleeds are performed at monthly intervals until antibody against OP-1 is detected in the serum using an ELISA assay. Then, the rabbit is boosted with 100 μ g of antigen and bled (15 ml per bleed) at days seven and ten after boosting.

15

Monoclonal antibody specific for a given morphogen may be prepared as follows. A mouse is given two injections of E. coli produced OP-1 monomer. The first injection contains 100 μ g of OP-1 in complete Freund's adjuvant and is given subcutaneously. The second injection contains 50 μ g of OP-1 in incomplete adjuvant and is given intraperitoneally. The mouse then receives a total of 230 μ g of OP-1 (amino acids 307-431 in SEQ ID NO:5) in four intraperitoneal injections at various times over an eight month period. One week prior to fusion, the mouse is boosted intraperitoneally with 100 μ g of OP-1 (307-431) and 30 μ g of the N-terminal peptide (Ser₂₉₃-Asn₃₀₉-Cys) conjugated through the added cysteine to bovine serum albumin with SMCC crosslinking agent. This boost was repeated five days (IP), four days (IP), three days (IP) and one day (IV) prior to fusion. The mouse spleen cells are then fused to myeloma (e.g., 653) cells at a ratio of 1:1 using PEG 1500 (Boeringer Mannheim), and the cell fusion is plated and screened for OP-1-specific antibodies using OP-1 (307-431) as antigen. The cell fusion and monoclonal screening then are according to standard

35

procedures well described in standard texts widely available in the art.

Example 4. Soluble Morphogen Complexes

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A currently preferred form of the morphogen useful in therapeutic formulations for systemic administration, having improved solubility in aqueous solutions and consisting essentially of amino acids, is a dimeric morphogenic protein comprising at least the 100 amino acid peptide sequence having the pattern of seven or more cysteine residues characteristic of the morphogen family complexed with a peptide comprising part or all of a pro region of a member of the morphogen family, or an allelic, species or other sequence variant thereof. Preferably, the dimeric morphogenic protein is complexed with two peptides. Also, the dimeric morphogenic protein preferably is noncovalently complexed with the pro region peptide or peptides. The pro region peptides also preferably comprise at least the N-terminal eighteen amino acids that define a given morphogen pro region. In a most preferred embodiment, peptides defining substantially the full length pro region are used.

25 Other soluble forms of morphogens include dimers of the uncleaved pro forms of these proteins, as well as "hemi-dimers" wherein one subunit of the dimer is an uncleaved pro form of the protein, and the other subunit comprises the mature form of the protein, including truncated forms thereof, preferably noncovalently associated with a cleaved pro domain peptide.

As described above, useful pro domains include the full length pro regions, as well as various truncated forms hereof, particularly truncated forms cleaved at proteolytic

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- Arg-Xaa-Xaa-Arg cleavage sites. For example, in OP-1, possible pro sequences include sequences defined by residues 30-292 (full length form); 48-292; and 158-292. Soluble OP-1 complex stability is enhanced when the pro region
- 5 comprises the full length form rather than a truncated form, such as the 48-292 truncated form, in that residues 30-47 show sequence homology to the N-terminal portions of other morphogens, and are believed to have particular utility in enhancing complex stability for all morphogens.
- 10 Accordingly, currently preferred pro sequences are those encoding the full length form of the pro region for a given morphogen. Other pro sequences contemplated to have utility include biosynthetic pro sequences, particularly those that incorporate a sequence derived from the N-terminal portion
- 15 of one or more morphogen pro sequences.

- As will be appreciated by those having ordinary skill in the art, useful sequences encoding the pro region may be obtained from genetic sequences encoding known morphogens.
- 20 Alternatively, chimeric pro regions can be constructed from the sequences of one or more known morphogens. Still another option is to create a synthetic sequence variant of one or more known pro region sequences.

- 25 In another preferred aspect, useful pro region peptides include polypeptide chains comprising an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions with a DNA or RNA sequence encoding at least the N-terminal eighteen amino acids of the pro region
- 30 sequence for OP1 or OP2, e.g., nucleotides 136-192 and 152-211 of Seq. ID No. 16 and 20, respectively.

4.1 Isolation of Soluble morphogen complex from conditioned media or body fluid

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Morphogens are expressed from mammalian cells as soluble complexes. Typically, however the complex is disassociated during purification, generally by exposure to denaturants often added to the purification solutions, such as

5 detergents, alcohols, organic solvents, chaotropic agents and compounds added to reduce the pH of the solution. Provided below is a currently preferred protocol for purifying the soluble proteins from conditioned media (or, optionally, a body fluid such as serum, cerebro-spinal or

10 peritoneal fluid), under non-denaturing conditions. The method is rapid, reproducible and yields isolated soluble morphogen complexes in substantially pure form.

Soluble morphogen complexes can be isolated from

15 conditioned media using a simple, three step chromatographic protocol performed in the absence of denaturants. The protocol involves running the media (or body fluid) over an affinity column, followed by ion exchange and gel filtration chromatographies. The affinity column described below is a

20 Zn-IMAC column. The present protocol has general applicability to the purification of a variety of morphogens, all of which are anticipated to be isolatable using only minor modifications of the protocol described below. An alternative protocol also envisioned to have

25 utility an immunoaffinity column, created using standard procedures and, for example, using antibody specific for a given morphogen pro domain (complexed, for example, to a protein A-conjugated Sepharose column.) Protocols for developing immunoaffinity columns are well described in the

30 art, (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly sections VII and XI.)

In this experiment OP-1 was expressed in mammalian CHO

35 (chinese hamster ovary) cells as described in the art (see,

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for example, international application US90/05903 (WO91/05802).) The CHO cell conditioned media containing 0.5% FBS was initially purified using Immobilized Metal-Ion Affinity Chromatography (IMAC). The soluble OP-1 complex
5 from conditioned media binds very selectively to the Zn-IMAC resin and a high concentration of imidazole (50 mM imidazole, pH 8.0) is required for the effective elution of the bound complex. The Zn-IMAC step separates the soluble OP-1 from the bulk of the contaminating serum proteins that
10 elute in the flow through and 35 mM imidazole wash fractions. The Zn-IMAC purified soluble OP-1 is next applied to an S-Sepharose cation-exchange column equilibrated in 20 mM NaPO_4 (pH 7.0) with 50 mM NaCl. This S-Sepharose step serves to further purify and concentrate
15 the soluble OP-1 complex in preparation for the following gel filtration step. The protein was applied to a Sephacryl S-200HR column equilibrated in TBS. Using substantially the same protocol, soluble morphogens also may be isolated from one or more body fluids, including serum, cerebro-spinal
20 fluid or peritoneal fluid.

IMAC was performed using Chelating-Sepharose (Pharmacia) that had been charged with three column volumes of 0.2 M ZnSO_4 . The conditioned media was titrated to pH 7.0 and
25 applied directly to the Zn-IMAC resin equilibrated in 20 mM HEPES (pH 7.0) with 500 mM NaCl. The Zn-IMAC resin was loaded with 80 mL of starting conditioned media per mL of resin. After loading, the column was washed with equilibration buffer and most of the contaminating proteins
30 were eluted with 35 mM imidazole (pH 7.0) in equilibration buffer. The soluble OP-1 complex then is eluted with 50 mM imidazole (pH 8.0) in 20 mM HEPES and 500 mM NaCl.

The 50 mM imidazole eluate containing the soluble OP-1
35 complex was diluted with nine volumes of 20 mM NaPO_4 (pH

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- 7.0) and applied to an S-Sepharose (Pharmacia) column equilibrated in 20 mM NaPO_4 (pH 7.0) with 50 mM NaCl. The S-Sepharose resin was loaded with an equivalent of 800 mL of starting conditioned media per mL of resin. After loading
- 5 the S-Sepharose column was washed with equilibration buffer and eluted with 100 mM NaCl followed by 300 mM and 500 mM NaCl in 20 mM NaPO_4 (pH 7.0). The 300 mM NaCl pool was further purified using gel filtration chromatography. Fifty
- 10 mls of the 300 mM NaCl eluate was applied to a 5.0 X 90 cm Sephacryl S-200HR (Pharmacia) equilibrated in Tris buffered saline (TBS), 50 mM Tris, 150 mM NaCl (pH 7.4). The column was eluted at a flow rate of 5 mL/minute collecting 10 mL fractions. The apparent molecular of the soluble OP-1 was determined by comparison to protein molecular weight
- 15 standards (alcohol dehydrogenase (ADH, 150 kDa), bovine serum albumin (BSA, 68 kDa), carbonic anhydrase (CA, 30 kDa) and cytochrome C (cyt C, 12.5 kDa). The purity of the S-200 column fractions was determined by separation on standard 15% polyacrylamide SDS gels stained with coomassie blue.
- 20 The identity of the mature OP-1 and the pro-domain was determined by N-terminal sequence analysis after separation of the mature OP-1 from the pro-domain using standard reverse phase C18 HPLC.
- 25 The soluble OP-1 complex elutes with an apparent molecular weight of 110 kDa. This agrees well with the predicted composition of the soluble OP-1 complex with one mature OP-1 dimer (35-36 kDa) associated with two pro-domains (39 kDa each). Purity of the final complex can
- 30 be verified by running the appropriate fraction in a reduced 15% polyacrylamide gel.

The complex components can be verified by running the complex-containing fraction from the S-200 or S-200HR

35 columns over a reverse phase C18 HPLC column and eluting in

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an acetonitrile gradient (in 0.1% TFA), using standard procedures. The complex is dissociated by this step, and the pro domain and mature species elute as separate species. These separate species then can be subjected to N-terminal sequencing using standard procedures (see, for example, 5 Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly pp. 602-613), and the identity of the isolated 36kD, 39kDa proteins confirmed as mature morphogen and isolated, cleaved pro domain, 10 respectively. N-terminal sequencing of the isolated pro domain from mammalian cell produced OP-1 revealed 2 forms of the pro region, the intact form (beginning at residue 30 of Seq. ID No. 16) and a truncated form, (beginning at residue 48 of Seq. ID No. 16.) N-terminal sequencing of the 15 polypeptide subunit of the isolated mature species reveals a range of N-termini for the mature sequence, beginning at residues 293, 300, 313, 315, 316, and 318, of Seq. ID No. 16, all of which are active as demonstrated by the standard bone induction assay.

20

4.2 In Vitro Soluble Morphogen Complex Formation

As an alternative to purifying soluble complexes from culture media or a body fluid, soluble complexes may be 25 formulated from purified pro domains and mature dimeric species. Successful complex formation apparently requires association of the components under denaturing conditions sufficient to relax the folded structure of these molecules, without affecting disulfide bonds. Preferably, the 30 denaturing conditions mimic the environment of an intracellular vesicle sufficiently such that the cleaved pro domain has an opportunity to associate with the mature dimeric species under relaxed folding conditions. The concentration of denaturant in the solution then is 35 decreased in a controlled, preferably step-wise manner, so

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as to allow proper refolding of the dimer and pro regions while maintaining the association of the pro domain with the dimer. Useful denaturants include 4-6M urea or guanidine hydrochloride (GuHCl), in buffered solutions of pH 4-10, preferably pH 6-8. The soluble complex then is formed by controlled dialysis or dilution into a solution having a final denaturant concentration of less than 0.1-2M urea or GuHCl, preferably 1-2 M urea or GuHCl, which then preferably can be diluted into a physiological buffer. Protein purification/renaturing procedures and considerations are well described in the art, and details for developing a suitable renaturing protocol readily can be determined by one having ordinary skill in the art. One useful text one the subject is Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly section V. Complex formation also may be aided by addition of one or more chaperone proteins.

4.3 Stability of Soluble Morphogen Complexes

The stability of the highly purified soluble morphogen complex in a physiological buffer, e.g., tris-buffered saline (TBS) and phosphate-buffered saline (PBS), can be enhanced by any of a number of means. Currently preferred is by means of a pro region that comprises at least the first 18 amino acids of the pro sequence (e.g., residues 30-47 of Seq. ID NO. 16 for OP-1), and preferably is the full length pro region. Residues 30-47 show sequence homology to the N-terminal portion of other morphogens and are believed to have particular utility in enhancing complex stability for all morphogens. Other useful means for enhancing the stability of soluble morphogen complexes include three classes of additives. These additives include basic amino acids (e.g., L-arginine, lysine and betaine); nonionic detergents (e.g., Tween 80 or Nonidet P-120); and

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carrier proteins (e.g., serum albumin and casein). Useful concentrations of these additives include 1-100 mM, preferably 10-70 mM, including 50 mM, basic amino acid;, 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (v/v) 5 nonionic detergent;, and 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (w/v) carrier protein.

The invention may be embodied in other specific forms without departing from the spirit or essential 10 characteristics thereof. The present embodiments are therefore to be considered in all respects as illustrative and not restrictive, the scope of the invention being indicated by the appended claims rather than by the foregoing description, and all changes which come within the 15 meaning and range of equivalency of the claims are therefore intended to be embraced therein.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5

(i) APPLICANT:

- (A) NAME: CREATIVE BIOMOLECULES, INC.
- (B) STREET: 45 SOUTH STREET
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- 10 (D) STATE: MA
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- (F) POSTAL CODE (ZIP): 01748
- (G) TELEPHONE: 1-508-435-9001
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- 15 (I) TELEX:

(ii) TITLE OF INVENTION: MORPHOGEN-INDUCED PERIODONTAL
TISSUE REGENERATION

20 (iii) NUMBER OF SEQUENCES: 33

(iv) CORRESPONDENCE ADDRESS:

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- (F) ZIP: 01748

30 (v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- 35 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- 40 (C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

- (A) APPLICATION NUMBER:
- (B) FILING DATE:

45 (viii) ATTORNEY/AGENT INFORMATION:

- (A) NAME: KELLEY ESQ, ROBIN D.
- (B) REGISTRATION NUMBER: 34,637
- (C) REFERENCE/DOCKET NUMBER: CRP-067

50 (ix) TELECOMMUNICATION INFORMATION:

- (A) TELEPHONE: 617/248-7477
- (B) TELEFAX: 617/248-7100

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 97 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

(ix) FEATURE:

- 15 (A) NAME/KEY: Protein
 (B) LOCATION: 1..97
 (D) OTHER INFORMATION: /label= GENERIC-SEQ1
 /note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES
 ONE OF THE 20 NATURALLY-OCCURING L-ISOMER, A-AMINO
 ACIDS, OR A DERIVATIVE THEREOF."

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

25 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 1 5 10 15
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa
 20 25 30
 30 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 35 40 45
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa
 50 55 60
 35 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 65 70 75 80
 40 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
 85 90 95
 Xaa

45 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- 50 (A) LENGTH: 97 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(ix) FEATURE:

- 5 (A) NAME/KEY: Protein
(B) LOCATION: 1..97
(D) OTHER INFORMATION: /label= GENERIC-SEQ2
/note= "WHEREIN EACH XAA INDEPENDENTLY INDICATES
ONE OF THE 20 NATURALLY OCCURRING L-ISOMER A-AMINO
ACIDS, OR A DERIVATIVE THEREOF."

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

15 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Xaa Xaa Cys Xaa Xaa Xaa
20 20 25 30
Xaa Xaa Xaa Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
25 35 40 45
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Xaa
50 55 60
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
25 65 70 75 80
Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Xaa Cys
30 85 90 95
Xaa

35 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- 40 (A) LENGTH: 97 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45

(ix) FEATURE:

- 50 (A) NAME/KEY: Protein
(B) LOCATION: 1..97
(D) OTHER INFORMATION: /label= GENERIC-SEQ3
/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
AS DEFINED IN THE SPECIFICATION."

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

5 Leu Tyr Val Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa Xaa Ala
 1 5 10 15
 Pro Xaa Gly Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
 20 25 30
 10 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa Leu
 35 40 45
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro
 50 55 60
 15 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 65 70 75 80
 20 Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Gly Cys
 85 90 95
 Xaa

25 (2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

35

(ix) FEATURE:

- 40 (A) NAME/KEY: Protein
 (B) LOCATION: 1..102
 (D) OTHER INFORMATION: /label= GENERIC-SEQ4
 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
 FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
 AS DEFINED IN THE SPECIFICATION."

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Xaa Xaa Xaa Xaa Leu Tyr Val Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
 1 5 10 15
 50 Xaa Trp Xaa Xaa Ala Pro Xaa Gly Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
 20 25 30

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Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
 35 40 45
 Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 5 50 55 60
 Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
 65 70 75 80
 10 Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val
 85 90 95
 Xaa Xaa Cys Gly Cys Xaa
 100
 15

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 139 amino acids
 20 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein
 25 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
 (F) TISSUE TYPE: HIPPOCAMPUS
 30 (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..139
 (D) OTHER INFORMATION: /label= hOPl-MATURE

35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
 1 5 10 15
 40 Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser Ser
 20 25 30
 Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
 45 35 40 45
 Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
 50 55 60
 50 Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
 65 70 75 80

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Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
 85 90 95

5 Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
 100 105 110

 Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
 115 120 125

10 Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 130 135

(2) INFORMATION FOR SEQ ID NO:6:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 139 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: MURIDAE
 (F) TISSUE TYPE: EMBRYO

25 (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..139
 (D) OTHER INFORMATION: /label= MOP1-MATURE

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

35 Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
 1 5 10 15

 Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser
 20 25 30

40 Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
 35 40 45

45 Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
 50 55 60

 Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
 65 70 75 80

50 Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
 85 90 95

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Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
 100 105 110
 5 Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
 115 120 125
 Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 130 135

10 (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 139 amino acids
 (B) TYPE: amino acid
 15 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein
 20 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS
 (F) TISSUE TYPE: HIPPOCAMPUS
 25 (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..139
 (D) OTHER INFORMATION: /label= HOP2-MATURE

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu
 1 5 10 15
 35 Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser
 20 25 30
 His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln
 35 40 45
 40 Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
 50 55 60
 45 Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn
 65 70 75 80
 Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
 85 90 95
 50 Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
 100 105 110

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Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
 115 120 125

5 Arg Asn Met Val Val Lys Ala Cys Gly Cys His
 130 135

(2) INFORMATION FOR SEQ ID NO:8:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
 (F) TISSUE TYPE: EMBRYO

20

(ix) FEATURE:

- (A) NAME/KEY: Protein
 (B) LOCATION: 1..139
 (D) OTHER INFORMATION: /label= MOP2-MATURE

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

30 Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu
 1 5 10 15

Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser
 20 25 30

35 Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg
 35 40 45

40 Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala
 50 55 60

Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn
 65 70 75 80

45 Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro
 85 90 95

Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr
 100 105 110

50 Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His
 115 120 125

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Arg Asn Met Val Val Lys Ala Cys Gly Cys His
130 135

(2) INFORMATION FOR SEQ ID NO:9:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 101 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 10 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
 15 (A) ORGANISM: bovinæ
- (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..101
 20 (D) OTHER INFORMATION: /label= CBMP-2A-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

25 Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
 1 5 10 15

Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly
 20 25 30

30 Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
 35 35 40 45

Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala
 50 55 60

Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
 65 70 75 80

40 Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu
 85 90 95

Gly Cys Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:10:

- 50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 101 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

5 (A) ORGANISM: HOMO SAPIENS
(F) TISSUE TYPE: hippocampus

(ix) FEATURE:

10 (A) NAME/KEY: Protein
(B) LOCATION: 1..101
(D) OTHER INFORMATION: /label= CBMP-2B-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

15 Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn
1 5 10 15
Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly
20 20 25 30
Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala
35 40 45
Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala
25 50 55 60
Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp
65 70 75 80
30 Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu
85 90 95
Gly Cys Gly Cys Arg
100

35

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45

(vi) ORIGINAL SOURCE:

(A) ORGANISM: DROSOPHILA MELANOGASTER

(ix) FEATURE:

50 (A) NAME/KEY: Protein
(B) LOCATION: 1..101
(D) OTHER INFORMATION: /label= DPP-FX

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

5 Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp
 1 5 10 15
 Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly
 20 25 30
 10 Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala
 35 40 45
 Val Val Gln Thr Leu Val Asn Asn Asn Asn Pro Gly Lys Val Pro Lys
 50 55 60
 15 Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu
 65 70 75 80
 20 Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val
 85 90 95
 Val Gly Cys Gly Cys Arg
 100

25 (2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 30 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: protein

 35 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: XENOPUS

 (ix) FEATURE:
 (A) NAME/KEY: Protein
 40 (B) LOCATION: 1..102
 (D) OTHER INFORMATION: /label= VGL-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

45 Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly Trp Gln
 1 5 10 15
 50 Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly
 20 25 30
 Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn His Ala
 35 40 45

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Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile Pro Leu
 50 55 60

5 Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu Phe Tyr
 65 70 75 80

Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met Ala Val
 85 90 95

10 Asp Glu Cys Gly Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:13:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 25 (A) ORGANISM: MURIDAE

(ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..102
 30 (D) OTHER INFORMATION: /label= VGR-1-FX

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

35 Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Val Gly Trp Gln
 1 5 10 15

Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
 20 25 30

40 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 35 40 45

45 Ile Val Gln Thr Leu Val His Val Met Asn Pro Glu Tyr Val Pro Lys
 50 55 60

Pro Cys Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val Leu Tyr Phe
 65 70 75 80

50 Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
 85 90 95

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Arg Ala Cys Gly Cys His
100

(2) INFORMATION FOR SEQ ID NO:14:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 106 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 10 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- 15 (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: Homo sapiens
 20 (F) TISSUE TYPE: brain
- (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..106
 25 (D) OTHER INFORMATION: /note= "GDF-1 (fx)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

30 Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp His
 1 5 10 15

Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly
 20 25 30

35 Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala
 35 40 45

40 Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro Gly
 50 55 60

Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile Ser
 65 70 75 80

45 Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr Glu
 85 90 95

Asp Met Val Val Asp Glu Cys Gly Cys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: peptide

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:
 Cys Xaa Xaa Xaa Xaa
 1 5

15 (2) INFORMATION FOR SEQ ID NO:16:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1822 base pairs
 (B) TYPE: nucleic acid
 20 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: cDNA
 25 (iii) HYPOTHETICAL: NO
 (iv) ANTI-SENSE: NO
 (vi) ORIGINAL SOURCE:
 30 (A) ORGANISM: HOMO SAPIENS
 (F) TISSUE TYPE: HIPPOCAMPUS
 (ix) FEATURE:
 35 (A) NAME/KEY: CDS
 (B) LOCATION: 49..1341
 (C) IDENTIFICATION METHOD: experimental
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "OP1"
 /evidence= EXPERIMENTAL
 40 /standard_name= "OP1"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

45 GGTGCGGGCC CGGAGCCCGG AGCCCGGGTA GCGCGTAGAG CCGGCGCG ATG CAC GTG 57
 Met His Val
 1

CGC TCA CTG CGA GCT GCG GCG CCG CAC AGC TTC GTG GCG CTC TGG GCA 105
 50 Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala
 5 10 15

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	CCC CTG TTC CTG CTG CGC TCC GCC CTG GCC GAC TTC AGC CTG GAC AAC	153
	Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn	
	20 25 30 35	
5	GAG GTG CAC TCG AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG	201
	Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg	
	40 45 50	
10	CGG GAG ATG CAG CGC GAG ATC CTC TCC ATT TTG GGC TTG CCC CAC CGC	249
	Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg	
	55 60 65	
15	CCG CGC CCG CAC CTC CAG GGC AAG CAC AAC TCG GCA CCC ATG TTC ATG	297
	Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met	
	70 75 80	
20	CTG GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG GGC GGC GGG CCC GGC	345
	Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly Gly Pro Gly	
	85 90 95	
25	GGC CAG GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC	393
	Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly	
	100 105 110 115	
30	CCC CCT CTG GCC AGC CTG CAA GAT AGC CAT TTC CTC ACC GAC GCC GAC	441
	Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp	
	120 125 130	
35	ATG GTC ATG AGC TTC GTC AAC CTC GTG GAA CAT GAC AAG GAA TTC TTC	489
	Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe	
	135 140 145	
40	CAC CCA CGC TAC CAC CAT CGA GAG TTC CGG TTT GAT CTT TCC AAG ATC	537
	His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile	
	150 155 160	
45	CCA GAA GGG GAA GCT GTC ACG GCA GCC GAA TTC CGG ATC TAC AAG GAC	585
	Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp	
	165 170 175	
50	TAC ATC CGG GAA CGC TTC GAC AAT GAG ACG TTC CGG ATC AGC GTT TAT	633
	Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile Ser Val Tyr	
	180 185 190 195	
55	CAG GTG CTC CAG GAG CAC TTG GGC AGG GAA TCG GAT CTC TTC CTG CTC	681
	Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu Phe Leu Leu	
	200 205 210	
60	GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC	729
	Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp	
	215 220 225	

	ATC	ACA	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAT	CCG	CGG	CAC	AAC	CTG	777
	Ile	Thr	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	
			230					235					240				
5	GGC	CTG	CAG	CTC	TCG	GTG	GAG	ACG	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	825
	Gly	Leu	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	
		245					250					255					
10	AAG	TTG	GCG	GGC	CTG	ATT	GGG	CGG	CAC	GGG	CCC	CAG	AAC	AAG	CAG	CCC	873
	Lys	Leu	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	Lys	Gln	Pro	
		260				265					270					275	
15	TTC	ATG	GTG	GCT	TTC	TTC	AAG	GCC	ACG	GAG	GTC	CAC	TTC	CGC	AGC	ATC	921
	Phe	Met	Val	Ala	Phe	Phe	Lys	Ala	Thr	Glu	Val	His	Phe	Arg	Ser	Ile	
					280					285					290		
20	CGG	TCC	ACG	GGG	AGC	AAA	CAG	CGC	AGC	CAG	AAC	CGC	TCC	AAG	ACG	CCC	969
	Arg	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	Lys	Thr	Pro	
				295					300					305			
	AAG	AAC	CAG	GAA	GCC	CTG	CGG	ATG	GCC	AAC	GTG	GCA	GAG	AAC	AGC	AGC	1017
	Lys	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu	Asn	Ser	Ser	
			310					315					320				
25	AGC	GAC	CAG	AGG	CAG	GCC	TGT	AAG	AAG	CAC	GAG	CTG	TAT	GTC	AGC	TTC	1065
	Ser	Asp	Gln	Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	
		325					330					335					
30	CGA	GAC	CTG	GGC	TGG	CAG	GAC	TGG	ATC	ATC	GCG	CCT	GAA	GGC	TAC	GCC	1113
	Arg	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala	
		340				345					350					355	
35	GCC	TAC	TAC	TGT	GAG	GGG	GAG	TGT	GCC	TTC	CCT	CTG	AAC	TCC	TAC	ATG	1161
	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn	Ser	Tyr	Met	
				360					365						370		
40	AAC	GCC	ACC	AAC	CAC	GCC	ATC	GTG	CAG	ACG	CTG	GTC	CAC	TTC	ATC	AAC	1209
	Asn	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Phe	Ile	Asn	
				375					380					385			
	CCG	GAA	ACG	GTG	CCC	AAG	CCC	TGC	TGT	GCG	CCC	ACG	CAG	CTC	AAT	GCC	1257
	Pro	Glu	Thr	Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln	Leu	Asn	Ala	
			390					395					400				
45	ATC	TCC	GTC	CTC	TAC	TTC	GAT	GAC	AGC	TCC	AAC	GTC	ATC	CTG	AAG	AAA	1305
	Ile	Ser	Val	Leu	Tyr	Phe	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys	Lys	
		405					410					415					
50	TAC	AGA	AAC	ATG	GTG	GTC	CGG	GCC	TGT	GGC	TGC	CAC	TAGCTCCTCC				1351
	Tyr	Arg	Asn	Met	Val	Val	Arg	Ala	Cys	Gly	Cys	His					
		420				425					430						
	GAGAAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CTTTGGCCAG																1411

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GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCCTC CCTATCCCCA ACTTTAAAGG 1471
 TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC 1531
 5 ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC 1591
 GCATAAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT 1651
 10 CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG 1711
 GCGGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC 1771
 CTGTAATAAA TGTACAATA AAACGAATGA ATGAAAAAAAA AAAAAAAAAA A 1822
 15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 431 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
 1 5 10 15
 30 Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
 20 25 30
 35 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
 35 40 45
 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
 50 55 60
 40 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
 65 70 75 80
 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly
 85 90 95
 45 Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser
 100 105 110
 50 Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr
 115 120 125
 Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys
 130 135 140

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Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu
 145 150 155 160
 5 Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile
 165 170 175
 Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile
 180 185 190
 10 Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu
 195 200 205
 Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu
 15 210 215 220
 Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg
 225 230 235 240
 20 His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser
 245 250 255
 Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn
 260 265 270
 25 Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe
 275 280 285
 Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser
 30 290 295 300
 Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu
 305 310 315 320
 35 Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr
 325 330 335
 Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu
 340 345 350
 40 Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn
 355 360 365
 Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His
 45 370 375 380
 Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln
 385 390 395 400
 50 Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile
 405 410 415

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Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:18:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1873 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 10 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- 15 (iv) ANTI-SENSE: NO
- (v) ORIGINAL SOURCE:
 (A) ORGANISM: MURIDAE
 20 (F) TISSUE TYPE: EMBRYO
- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 104..1393
 25 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 /product= "MOP1"
 /note= "MOP1 (CDNA)"

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG 60

CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC 115
 35 Met His Val Arg
 1

TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT 163
 40 Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro
 5 10 15 20

CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG 211
 Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu
 25 30 35

45 GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG 259
 Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg
 40 45 50

50 GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG 307
 Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro
 55 60 65

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	CGC	CCG	CAC	CTC	CAG	GGA	AAG	CAT	AAT	TCG	GCG	CCC	ATG	TTC	ATG	TTG	355
	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	Phe	Met	Leu	
	70						75					80					
5	GAC	CTG	TAC	AAC	GCC	ATG	GCG	GTG	GAG	GAG	AGC	GGG	CCG	GAC	GGA	CAG	403
	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Ser	Gly	Pro	Asp	Gly	Gln	
	85					90					95					100	
10	GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	CCC	CCT	451
	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	Pro	Pro	
					105					110					115		
15	TTA	GCC	AGC	CTG	CAG	GAC	AGC	CAT	TTC	CTC	ACT	GAC	GCC	GAC	ATG	GTC	499
	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	Met	Val	
				120					125					130			
20	ATG	AGC	TTC	GTC	AAC	CTA	GTG	GAA	CAT	GAC	AAA	GAA	TTC	TTC	CAC	CCT	547
	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	His	Pro	
			135					140					145				
25	CGA	TAC	CAC	CAT	CGG	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	CCC	GAG	595
	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	Pro	Glu	
	150					155						160					
30	GGC	GAA	CGG	GTG	ACC	GCA	GCC	GAA	TTC	AGG	ATC	TAT	AAG	GAC	TAC	ATC	643
	Gly	Glu	Arg	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	Tyr	Ile	
	165					170					175					180	
35	CGG	GAG	CGA	TTT	GAC	AAC	GAG	ACC	TTC	CAG	ATC	ACA	GTC	TAT	CAG	GTG	691
	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Gln	Ile	Thr	Val	Tyr	Gln	Val	
				185						190					195		
40	CTC	CAG	GAG	CAC	TCA	GGC	AGG	GAG	TCG	GAC	CTC	TTC	TTG	CTG	GAC	AGC	739
	Leu	Gln	Glu	His	Ser	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	Asp	Ser	
				200					205					210			
45	CGC	ACC	ATC	TGG	GCT	TCT	GAG	GAG	GGC	TGG	TTG	GTG	TTT	GAT	ATC	ACA	787
	Arg	Thr	Ile	Trp	Ala	Ser	Glu	Glu	Gly	Trp	Leu	Val	Phe	Asp	Ile	Thr	
			215					220					225				
50	GCC	ACC	AGC	AAC	CAC	TGG	GTG	GTC	AAC	CCT	CGG	CAC	AAC	CTG	GGC	TTA	835
	Ala	Thr	Ser	Asn	His	Trp	Val	Val	Asn	Pro	Arg	His	Asn	Leu	Gly	Leu	
	230					235						240					
55	CAG	CTC	TCT	GTG	GAG	ACC	CTG	GAT	GGG	CAG	AGC	ATC	AAC	CCC	AAG	TTG	883
	Gln	Leu	Ser	Val	Glu	Thr	Leu	Asp	Gly	Gln	Ser	Ile	Asn	Pro	Lys	Leu	
	245					250					255					260	
60	GCA	GGC	CTG	ATT	GGA	CGG	CAT	GGA	CCC	CAG	AAC	AAG	CAA	CCC	TTC	ATG	931
	Ala	Gly	Leu	Ile	Gly	Arg	His	Gly	Pro	Gln	Asn	Lys	Gln	Pro	Phe	Met	
					265					270					275		

	GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC	979
	Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser	
	280 285 290	
5	ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC	1027
	Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn	
	295 300 305	
10	CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC	1075
	Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp	
	310 315 320	
15	CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC	1123
	Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp	
	325 330 335 340	
20	CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC	1171
	Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr	
	345 350 355	
25	TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC	1219
	Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala	
	360 365 370	
30	ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC	1267
	Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp	
	375 380 385	
35	ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT	1315
	Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser	
	390 395 400	
40	GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC GAC CTG AAG AAG TAC AGA	1363
	Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Asp Leu Lys Lys Tyr Arg	
	405 410 415 420	
45	AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG	1413
	Asn Met Val Val Arg Ala Cys Gly Cys His	
	425 430	
50	ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG	1473
	CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG	1533
	AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT	1593
	GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT	1653
	GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCTGGC GCTCTGAGTC TTTGAGGAGT	1713
	AATCGCAAGC CTCGTTGAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GCGGCTGGCG	1773
	TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT	1833

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GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC

1873

5 (2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 430 amino acids

(B) TYPE: amino acid

10 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

15 Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala
1 5 10 15

20 Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser
20 20 25 30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser
35 40 45

25 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu
50 55 60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro
65 70 75 80

30 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly
85 90 95

35 Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr
100 105 110

Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp
115 120 125

40 Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu
130 135 140

Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser
145 150 155 160

45 Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr
165 170 175

50 Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr
180 185 190

Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe
195 200 205

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Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val
 210 215 220
 5 Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His
 225 230 235 240
 Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile
 245 250 255
 10 Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys
 260 265 270
 Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg
 15 275 280 285
 Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys
 290 295 300
 20 Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn
 305 310 315 320
 Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val
 325 330 335
 25 Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly
 340 345 350
 Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser
 30 355 360 365
 Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe
 370 375 380
 35 Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu
 385 390 395 400
 Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Asp Leu
 40 405 410 415
 Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His
 420 425 430

(2) INFORMATION FOR SEQ ID NO:20:

45

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1723 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

50

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

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(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens
(F) TISSUE TYPE: HIPPOCAMPUS

5 (ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 490..1696
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
/product= "hOP2-PP"
/note= "hOP2 (cDNA)"

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

15	GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA	60
	GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCAGG AGGCGCTGGA GCAACAGCTC	120
	CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCATC GCCCTGCGC TGCTCGGACC	180
20	GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT	240
	CCGCAGAGTA GCGCCGGCCT CGAGGCGGTG GCGTCCCGT CCTCTCCGTC CAGGAGCCAG	300
25	GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC	360
	CGCCCCGCCC CGCCGCCCCG CGCCGCCCCA GCGCAGCTC CTTGCCGTC GGGCGTCCCC	420
	AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC	480
30	CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG	528
	Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu	
	1 5 10	
35	GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC	576
	Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro	
	15 20 25	
	GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG	624
40	Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln	
	30 35 40 45	
	CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC	672
45	Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg	
	50 55 60	
	GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG	720
	Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met	
	65 70 75	
50	CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG	768
	Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala	
	80 85 90	

	CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT	816
	Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val	
	95 100 105	
5	AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG	864
	Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp	
	110 115 120 125	
10	AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC	912
	Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val	
	130 135 140	
15	ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC	960
	Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu	
	145 150 155	
20	AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC	1008
	Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser	
	160 165 170	
25	AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT	1056
	Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala	
	175 180 185	
30	GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC	1104
	Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys	
	190 195 200 205	
35	TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG	1152
	Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu	
	210 215 220	
40	ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT	1200
	Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly	
	225 230 235	
45	CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG	1248
	Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg	
	240 245 250	
50	GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG AGG	1296
	Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg	
	255 260 265	
55	AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC	1344
	Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu	
	270 275 280 285	
60	CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC	1392
	Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys	
	290 295 300	

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CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC 1440
 Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp
 305 310 315

5 TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG 1488
 Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu
 320 325 330

10 TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC 1536
 Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile
 335 340 345

15 CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG 1584
 Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala
 350 355 360 365

TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC 1632
 Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp
 370 375 380

20 AGC AGC AAC AAC GTC ATC CTG CGC AAA GCC CGC AAC ATG GTG GTC AAG 1680
 Ser Ser Asn Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys
 385 390 395

25 GCC TGC GGC TGC CAC T GAGTCAGCCC GCCCAGCCCT ACTGCAG 1723
 Ala Cys Gly Cys His
 400

30 (2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 402 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

40 Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1 5 10 15

45 Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro
 20 25 30

Gln Arg Arg Leu Gly Ala Arg Glu Arg Asp Val Gln Arg Glu Ile
 35 40 45

50 Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro
 50 55 60

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Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu
 65 70 75 80
 Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu
 5 85 90 95
 Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val
 100 105 110
 10 Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe
 115 120 125
 Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala
 130 135 140
 15 Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr
 145 150 155 160
 Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu
 20 165 170 175
 Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu
 180 185 190
 25 Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu
 195 200 205
 Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp
 210 215 220
 30 Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala
 225 230 235 240
 Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro
 35 245 250 255
 Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln
 260 265 270
 40 Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu Pro Gly Ile
 275 280 285
 Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys Arg Arg His
 290 295 300
 45 Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp Trp Val Ile
 305 310 315 320
 Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ser Phe
 50 325 330 335
 Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser
 340 345 350

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Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala Cys Cys Ala
 355 360 365
 5 Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn
 370 375 380
 Asn Val Ile Leu Arg Lys Ala Arg Asn Met Val Val Lys Ala Cys Gly
 385 390 395 400
 10 Cys His

(2) INFORMATION FOR SEQ ID NO:22:

15

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1926 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 20 (D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE
 (F) TISSUE TYPE: EMBRYO

25

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 93..1289
 (D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"
 30 /product= "mOP2-PP"
 /note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

35

GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC CCGACCAGCT 60

ACCAGTGGAT GCGCGCCGCG TGAAAGTCCG AG ATG GCT ATG CGT CCC GGG CCA 113

Met Ala Met Arg Pro Gly Pro
 1 5

40

CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC GGC CAC GGT 161

Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly His Gly
 10 15 20

45

CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA GCG CGC GAG 209

Pro Arg Pro Pro His Thr Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu
 25 30 35

50 CGC CGC GAC ATG CAG CGT GAA ATC CTG GCG GTG CTC GGG CTA CCG GGA 257

Arg Arg Asp Met Gln Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly
 40 45 50 55

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	CGG CCC CGA CCC CGT GCA CAA CCC GCC GCT GCC CGG CAG CCA GCG TCC	305
	Arg Pro Arg Pro Arg Ala Gln Pro Ala Ala Arg Gln Pro Ala Ser	
	60 65 70	
5	GCG CCC CTC TTC ATG TTG GAC CTA TAC CAC GCC ATG ACC GAT GAC GAC	353
	Ala Pro Leu Phe Met Leu Asp Leu Tyr His Ala Met Thr Asp Asp Asp	
	75 80 85	
10	GAC GGC GGG CCA CCA CAG GCT CAC TTA GGC CGT GCC GAC CTG GTC ATG	401
	Asp Gly Gly Pro Pro Gln Ala His Leu Gly Arg Ala Asp Leu Val Met	
	90 95 100	
15	AGC TTC GTC AAC ATG GTG GAA CGC GAC CGT ACC CTG GGC TAC CAG GAG	449
	Ser Phe Val Asn Met Val Glu Arg Asp Arg Thr Leu Gly Tyr Gln Glu	
	105 110 115	
20	CCA CAC TGG AAG GAA TTC CAC TTT GAC CTA ACC CAG ATC CCT GCT GGG	497
	Pro His Trp Lys Glu Phe His Phe Asp Leu Thr Gln Ile Pro Ala Gly	
	120 125 130 135	
25	GAG GCT GTC ACA GCT GCT GAG TTC CGG ATC TAC AAA GAA CCC AGC ACC	545
	Glu Ala Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Glu Pro Ser Thr	
	140 145 150	
30	CAC CCG CTC AAC ACA ACC CTC CAC ATC AGC ATG TTC GAA GTG GTC CAA	593
	His Pro Leu Asn Thr Thr Leu His Ile Ser Met Phe Glu Val Val Gln	
	155 160 165	
35	GAG CAC TCC AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG	641
	Glu His Ser Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr	
	170 175 180	
40	CTC CGA TCT GGG GAC GAG GGC TGG CTG GTG CTG GAC ATC ACA GCA GCC	689
	Leu Arg Ser Gly Asp Glu Gly Trp Leu Val Leu Asp Ile Thr Ala Ala	
	185 190 195	
45	AGT GAC CGA TGG CTG CTG AAC CAT CAC AAG GAC CTG GGA CTC CGC CTC	737
	Ser Asp Arg Trp Leu Leu Asn His His Lys Asp Leu Gly Leu Arg Leu	
	200 205 210 215	
50	TAT GTG GAA ACC GCG GAT GGG CAC AGC ATG GAT CCT GGC CTG GCT GGT	785
	Tyr Val Glu Thr Ala Asp Gly His Ser Met Asp Pro Gly Leu Ala Gly	
	220 225 230	
55	CTG CTT GGA CGA CAA GCA CCA CGC TCC AGA CAG CCT TTC ATG GTA ACC	833
	Leu Leu Gly Arg Gln Ala Pro Arg Ser Arg Gln Pro Phe Met Val Thr	
	235 240 245	
60	TTC TTC AGG GCC AGC CAG AGT CCT GTG CGG GCC CCT CGG GCA GCG AGA	881
	Phe Phe Arg Ala Ser Gln Ser Pro Val Arg Ala Pro Arg Ala Ala Arg	
	250 255 260	

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	CCA CTG AAG AGG AGG CAG CCA AAG AAA ACG AAC GAG CTT CCG CAC CCC	929
	Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu Pro His Pro	
	265 270 275	
5	AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC CGC GGC AGA	977
	Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly Arg	
	280 285 290 295	
10	GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT GAC CTT GGC	1025
	Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly	
	300 305 310	
15	TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC TGT	1073
	Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys	
	315 320 325	
	GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC AAC	1121
	Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn	
	330 335 340	
20	CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC	1169
	His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val	
	345 350 355	
25	CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG	1217
	Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu	
	360 365 370 375	
30	TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG	1265
	Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met	
	380 385 390	
35	GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCG CCCAGCATCC TGCTTCTACT	1319
	Val Val Lys Ala Cys Gly Cys His	
	395	
	ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT	1379
	CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCTGCTA AAATTCTGGT	1439
40	CTTTCCCACT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC CTCTCCATCC	1499
	TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT	1559
45	CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC CTCAGCCCAC	1619
	AATGGCAAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTC TAAACTAGAT GATCTGGGCT	1679
	CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTITAGGT ATAACAGACA CATACTTA	1739
50	GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAATCAGAG	1799
	CCAGGTATAG CGGTGCATGT CATTAAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAATCT	1859

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CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAAAC 1919

GGAATTC 1926

5

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 399 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met Ala Met Arg Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys
 1 5 10 15

20 Ala Leu Gly Gly Gly His Gly Pro Arg Pro Pro His Thr Cys Pro Gln
 20 25 30

Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Met Gln Arg Glu Ile Leu
 25 35 40 45

Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Gln Pro Ala
 50 55 60

30 Ala Ala Arg Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr
 65 70 75 80

His Ala Met Thr Asp Asp Asp Asp Gly Gly Pro Pro Gln Ala His Leu
 85 90 95

35 Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val Glu Arg Asp
 100 105 110

Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp
 40 115 120 125

Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg
 130 135 140

45 Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile
 145 150 155 160

Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu
 165 170 175

50 Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu
 180 185 190

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Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His
195 200 205

5 Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Ala Asp Gly His Ser
210 215 220

Met Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser
225 230 235 240

10 Arg Gln Pro Phe Met Val Thr Phe Phe Arg Ala Ser Gln Ser Pro Val
245 250 255

Arg Ala Pro Arg Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys
260 265 270

15 Thr Asn Glu Leu Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp
275 280 285

Gly His Gly Ser Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr
20 290 295 300

Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln
305 310 315 320

25 Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp
325 330 335

Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His
340 345 350

30 Leu Met Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys
355 360 365

Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile
35 370 375 380

Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His
385 390 395

40 (2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1368 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

50

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..1368

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

5	ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC	48
	Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser	
	1 5 10 15	
10	CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG	96
	Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro	
	20 25 30	
15	GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC	144
	Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp	
	35 40 45	
20	CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC GAC AAG CTG GAC GTC	192
	Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val	
	50 55 60	
25	TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC	240
	Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His	
	65 70 75 80	
30	CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG	288
	Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu	
	85 90 95	
35	CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG	336
	Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln	
	100 105 110	
40	GAT GAG GAC GAC GAC TAC GAA CGC GGC CAT CGG TCC AGG AGG AGC GCC	384
	Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala	
	115 120 125	
45	GAC CTC GAG GAG GAT GAG GGC GAG CAG CAG AAG AAC TTC ATC ACC GAC	432
	Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp	
	130 135 140	
50	CTG GAC AAG CGG GCC ATC GAC GAG AGC GAC ATC ATC ATG ACC TTC CTG	480
	Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu	
	145 150 155 160	
55	AAC AAG CGC CAC CAC AAT GTG GAC GAA CTG CGT CAC GAG CAC GGC CGT	528
	Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg	
	165 170 175	
60	CGC CTG TGG TTC GAC GTC TCC AAC GTG CCC AAC GAC AAC TAC CTG GTG	576
	Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val	
	180 185 190	

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	ATG GCC GAG CTG CGC ATC TAT CAG AAC GCC AAC GAG GGC AAG TGG CTG Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu	624
	195 200 205	
5	ACC GCC AAC AGG GAG TTC ACC ATC ACG GTA TAC GCC ATT GGC ACC GGC Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly	672
	210 215 220	
10	ACG CTG GGC CAG CAC ACC ATG GAG CCG CTG TCC TCG GTG AAC ACC ACC Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr	720
	225 230 235 240	
15	GGG GAC TAC GTG GGC TGG TTG GAG CTC AAC GTG ACC GAG GGC CTG CAC Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His	768
	245 250 255	
20	GAG TGG CTG GTC AAG TCG AAG GAC AAT CAT GGC ATC TAC ATT GGA GCA Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala	816
	260 265 270	
25	CAC GCT GTC AAC CGA CCC GAC CGC GAG GTG AAG CTG GAC GAC ATT GGA His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly	864
	275 280 285	
30	CTG ATC CAC CGC AAG GTG GAC GAC GAG TTC CAG CCC TTC ATG ATC GGC Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly	912
	290 295 300	
35	TTC TTC CGC GGA CCG GAG CTG ATC AAG GCG ACG GCC CAC AGC AGC CAC Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His	960
	305 310 315 320	
40	CAC AGG AGC AAG CGA AGC GCC AGC CAT CCA CGC AAG CGC AAG AAG TCG His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser	1008
	325 330 335	
45	GTG TCG CCC AAC AAC GTG CCG CTG CTG GAA CCG ATG GAG AGC ACG CGC Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg	1056
	340 345 350	
50	AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp	1104
	355 360 365	
55	CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser	1152
	370 375 380	
60	GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His	1200
	385 390 395 400	

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	GCG ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC	1248
	Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro	
	405 410 415	
5	AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC	1296
	Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr	
	420 425 430	
10	CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT	1344
	His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile	
	435 440 445	
15	GTG AAA TCC TGC GGG TGC CAT TGA	1368
	Val Lys Ser Cys Gly Cys His	
	450 455	

(2) INFORMATION FOR SEQ ID NO:25:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 455 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

30	Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser	
	1 5 10 15	
	Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro	
	20 25 30	
35	Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp	
	35 40 45	
40	Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val	
	50 55 60	
	Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His	
	65 70 75 80	
45	Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu	
	85 90 95	
	Leu Asp Val Tyr His Arg Ile Thr Ala Glu Glu Gly Leu Ser Asp Gln	
	100 105 110	
50	Asp Glu Asp Asp Asp Tyr Glu Arg Gly His Arg Ser Arg Arg Ser Ala	
	115 120 125	

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Asp Leu Glu Glu Asp Glu Gly Glu Gln Gln Lys Asn Phe Ile Thr Asp
 130 135 140
 5 Leu Asp Lys Arg Ala Ile Asp Glu Ser Asp Ile Ile Met Thr Phe Leu
 145 150 155 160
 Asn Lys Arg His His Asn Val Asp Glu Leu Arg His Glu His Gly Arg
 165 170 175
 10 Arg Leu Trp Phe Asp Val Ser Asn Val Pro Asn Asp Asn Tyr Leu Val
 180 185 190
 Met Ala Glu Leu Arg Ile Tyr Gln Asn Ala Asn Glu Gly Lys Trp Leu
 195 200 205
 15 Thr Ala Asn Arg Glu Phe Thr Ile Thr Val Tyr Ala Ile Gly Thr Gly
 210 215 220
 Thr Leu Gly Gln His Thr Met Glu Pro Leu Ser Ser Val Asn Thr Thr
 225 230 235 240
 Gly Asp Tyr Val Gly Trp Leu Glu Leu Asn Val Thr Glu Gly Leu His
 245 250 255
 25 Glu Trp Leu Val Lys Ser Lys Asp Asn His Gly Ile Tyr Ile Gly Ala
 260 265 270
 His Ala Val Asn Arg Pro Asp Arg Glu Val Lys Leu Asp Asp Ile Gly
 275 280 285
 30 Leu Ile His Arg Lys Val Asp Asp Glu Phe Gln Pro Phe Met Ile Gly
 290 295 300
 Phe Phe Arg Gly Pro Glu Leu Ile Lys Ala Thr Ala His Ser Ser His
 305 310 315 320
 His Arg Ser Lys Arg Ser Ala Ser His Pro Arg Lys Arg Lys Lys Ser
 325 330 335
 40 Val Ser Pro Asn Asn Val Pro Leu Leu Glu Pro Met Glu Ser Thr Arg
 340 345 350
 Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp
 355 360 365
 45 His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser
 370 375 380
 Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His
 385 390 395 400
 50 Ala Ile Val Gln Thr Leu Val His Leu Leu Glu Pro Lys Lys Val Pro
 405 410 415

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Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr
 420 425 430
 5 His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile
 435 440 445
 Val Lys Ser Cys Gly Cys His
 450 455
 10

(2) INFORMATION FOR SEQ ID NO:26:

15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 104 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 20 (ii) MOLECULE TYPE: protein
 25 (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..104
 (D) OTHER INFORMATION: /note= "BMP3"
 30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:
 Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser
 1 5 10 15
 35 Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly
 20 25 30
 Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala
 35 40 45
 40 Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile
 50 55 60
 Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu
 65 70 75 80
 45 Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met
 85 90 95
 50 Thr Val Glu Ser Cys Ala Cys Arg
 100

(2) INFORMATION FOR SEQ ID NO:27:

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(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS

(ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..102
 (D) OTHER INFORMATION: /note= "BMP5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

20	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln
	1				5					10					15	
	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala	Ala	Phe	Tyr	Cys	Asp	Gly
25			20					25						30		
	Glu	Cys	Ser	Phe	Pro	Leu	Asn	Ala	His	Met	Asn	Ala	Thr	Asn	His	Ala
			35					40					45			
	Ile	Val	Gln	Thr	Leu	Val	His	Leu	Met	Phe	Pro	Asp	His	Val	Pro	Lys
30		50					55					60				
	Pro	Cys	Cys	Ala	Pro	Thr	Lys	Leu	Asn	Ala	Ile	Ser	Val	Leu	Tyr	Phe
	65					70				75						80
35	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys	Lys	Tyr	Arg	Asn	Met	Val	Val
					85					90					95	
	Arg	Ser	Cys	Gly	Cys	His										
40					100											

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS

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(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /note= "BMP6"

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

10 Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln
 1 5 10 15
 Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
 20 25 30
 15 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
 35 40 45
 Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys
 50 55 60
 20 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe
 65 70 75 80
 25 Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val
 85 90 95
 Arg Ala Cys Gly Cys His
 100

30 (2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids

(B) TYPE: amino acid

35 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

40

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..102

(D) OTHER INFORMATION: /label= OPX

45

/note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
 FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
 AS DEFINED IN THE SPECIFICATION (SECTION II.B.2.)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

50

Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa
 1 5 10 15

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Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly
 20 25 30
 5 Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala
 35 40 45
 Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys
 50 55 60
 10 Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa
 65 70 75 80
 Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val
 85 90 95
 15 Xaa Ala Cys Gly Cys His
 100

(2) INFORMATION FOR SEQ ID NO:30:

20 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 97 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 25 (D) TOPOLOGY: linear

 (ii) MOLECULE TYPE: protein

 30 (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..97
 (D) OTHER INFORMATION: /label= GENERIC-SEQ5
 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
 35 FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
 AS DEFINED IN THE SPECIFICATION."

 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
 40 Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa Xaa Trp Xaa Xaa Xaa
 1 5 10 15
 Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
 45 20 25 30
 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa
 35 40 45
 50 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro
 50 55 60

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Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa
 65 70 75 80

5 Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Xaa Cys
 85 90 95

Xaa

10 (2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20

(ix) FEATURE:

- (A) NAME/KEY: Protein
 (B) LOCATION: 1..102
 (D) OTHER INFORMATION: /label= GENERIC-SEQ6
 25 /note= "WHEREIN EACH XAA IS INDEPENDENTLY SELECTED
 FROM A GROUP OF ONE OR MORE SPECIFIED AMINO ACIDS
 AS DEFINED IN THE SPECIFICATION. "

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
 1 5 10 15
 35 Xaa Trp Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
 20 25 30
 Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
 35 40 45
 40 Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
 50 55 60
 45 Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa
 65 70 75 80
 Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Met Xaa Val
 85 90 95
 50 Xaa Xaa Cys Xaa Cys Xaa
 100

(2) INFORMATION FOR SEQ ID NO:32:

- 114 -

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1247 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:
 (A) ORGANISM: HOMO SAPIENS
 (F) TISSUE TYPE: BRAIN

(ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 84..1199
 (D) OTHER INFORMATION: /product= "GDF-1"
 /note= "GDF-1 CDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

	GGGGACACCG GCCCGGCCCT CAGCCCACTG GTCCCGGGCC GCCGCGGACC CTGCGCACTC	60
25	TCTGGTCATC GCCTGGGAGG AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC Met Pro Pro Pro Gln Gln Gly Pro Cys 1 5	110
30	GGC CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC Gly His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro 10 15 20 25	158
35	CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC CAG Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu Gln 30 35 40	206
40	GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC CGG CCG Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu Arg Pro 45 50 55	254
45	GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC CCC CAG GAG Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp Pro Gln Glu 60 65 70	302
50	ACC AGG TCT GGC TCG CGG CGG ACG TCC CCA GGG GTC ACC CTG CAA CCG Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val Thr Leu Gln Pro 75 80 85	350
50	TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC ATC GTG CGC CAC ATC Cys His Val Glu Glu Leu Gly Val Ala Gly Asn Ile Val Arg His Ile 90 95 100 105	398

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	CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG GAG CCT GTC TCG GCC GCG	446
	Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser Glu Pro Val Ser Ala Ala	
	110 115 120	
5	GGG CAT TGC CCT GAG TGG ACA GTC GTC TTC GAC CTG TCG GCT GTG GAA	494
	Gly His Cys Pro Glu Trp Thr Val Val Phe Asp Leu Ser Ala Val Glu	
	125 130 135	
10	CCC GCT GAG CGC CCG AGC CGG GCC CGC CTG GAG CTG CGT TTC GCG GCG	542
	Pro Ala Glu Arg Pro Ser Arg Ala Arg Leu Glu Leu Arg Phe Ala Ala	
	140 145 150	
15	GCG GCG GCG GCA GCC CCG GAG GGC GGC TGG GAG CTG AGC GTG GCG CAA	590
	Ala Ala Ala Ala Ala Pro Glu Gly Gly Trp Glu Leu Ser Val Ala Gln	
	155 160 165	
20	GCG GGC CAG GGC GCG GGC GCG GAC CCC GGG CCG GTG CTG CTC CGC CAG	638
	Ala Gly Gln Gly Ala Gly Ala Asp Pro Gly Pro Val Leu Leu Arg Gln	
	170 175 180 185	
25	TTG GTG CCC GCC CTG GGG CCG CCA GTG CGC GCG GAG CTG CTG GGC GCC	686
	Leu Val Pro Ala Leu Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala	
	190 195 200	
30	GCT TGG GCT CGC AAC GCC TCA TGG CCG CGC AGC CTC CGC CTG GCG CTG	734
	Ala Trp Ala Arg Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu	
	205 210 215	
35	GCG CTA CGC CCC CGG GCC CCT GCC GCC TGC GCG CGC CTG GCC GAG GCC	782
	Ala Leu Arg Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala	
	220 225 230	
40	TCG CTG CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC	830
	Ser Leu Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala	
	235 240 245	
45	CGG CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC	878
	Arg Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly	
	250 255 260 265	
50	GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC GAG GTG GGC TGG	926
	Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp	
	270 275 280	
55	CAC CGC TGG GTC ATC GCG CCG CGC GGC TTC CTG GCC AAC TAC TGC CAG	974
	His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln	
	285 290 295	
60	GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG GGG CCG CCG	1022
	Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro	
	300 305 310	

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GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC GCG GCC GCC CCG 1070
 Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro
 315 320 325
 5 GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG CGC CTG TCG CCC ATC 1118
 Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile
 330 335 340 345
 TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC GTG GTG CTG CGG CAG TAT 1166
 10 Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr
 350 355 360
 GAG GAC ATG GTG GTG GAC GAG TGC GGC TGC CGC TAACCCGGGG CGGGCAGGGA 1219
 15 Glu Asp Met Val Val Asp Glu Cys Gly Cys Arg
 365 370
 CCGGGGCCCA ACAATAAATG CCGCGTGG 1247

20 (2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 372 amino acids
 (B) TYPE: amino acid
 25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

30 Met Pro Pro Pro Gln Gln Gly Pro Cys Gly His His Leu Leu Leu Leu
 1 5 10 15
 Leu Ala Leu Leu Leu Pro Ser Leu Pro Leu Thr Arg Ala Pro Val Pro
 35 20 25 30
 Pro Gly Pro Ala Ala Ala Leu Leu Gln Ala Leu Gly Leu Arg Asp Glu
 35 40 45
 40 Pro Gln Gly Ala Pro Arg Leu Arg Pro Val Pro Pro Val Met Trp Arg
 50 55 60
 Leu Phe Arg Arg Arg Asp Pro Gln Glu Thr Arg Ser Gly Ser Arg Arg
 65 70 75 80
 45 Thr Ser Pro Gly Val Thr Leu Gln Pro Cys His Val Glu Glu Leu Gly
 85 90 95
 Val Ala Gly Asn Ile Val Arg His Ile Pro Asp Arg Gly Ala Pro Thr
 50 100 105 110
 Arg Ala Ser Glu Pro Val Ser Ala Ala Gly His Cys Pro Glu Trp Thr
 115 120 125

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Val Val Phe Asp Leu Ser Ala Val Glu Pro Ala Glu Arg Pro Ser Arg
 130 135 140
 5 Ala Arg Leu Glu Leu Arg Phe Ala Ala Ala Ala Ala Ala Pro Glu
 145 150 155 160
 Gly Gly Trp Glu Leu Ser Val Ala Gln Ala Gly Gln Gly Ala Gly Ala
 165 170 175
 10 Asp Pro Gly Pro Val Leu Leu Arg Gln Leu Val Pro Ala Leu Gly Pro
 180 185 190
 15 Pro Val Arg Ala Glu Leu Leu Gly Ala Ala Trp Ala Arg Asn Ala Ser
 195 200 205
 Trp Pro Arg Ser Leu Arg Leu Ala Leu Ala Leu Arg Pro Arg Ala Pro
 210 215 220
 20 Ala Ala Cys Ala Arg Leu Ala Glu Ala Ser Leu Leu Leu Val Thr Leu
 225 230 235 240
 Asp Pro Arg Leu Cys His Pro Leu Ala Arg Pro Arg Arg Asp Ala Glu
 245 250 255
 25 Pro Val Leu Gly Gly Gly Pro Gly Gly Ala Cys Arg Ala Arg Arg Leu
 260 265 270
 Tyr Val Ser Phe Arg Glu Val Gly Trp His Arg Trp Val Ile Ala Pro
 275 280 285
 30 Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly Gln Cys Ala Leu Pro Val
 290 295 300
 35 Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala Leu Asn His Ala Val Leu
 305 310 315 320
 Arg Ala Leu Met His Ala Ala Ala Pro Gly Ala Ala Asp Leu Pro Cys
 325 330 335
 40 Cys Val Pro Ala Arg Leu Ser Pro Ile Ser Val Leu Phe Phe Asp Asn
 340 345 350
 45 Ser Asp Asn Val Val Leu Arg Gln Tyr Glu Asp Met Val Val Asp Glu
 355 360 365
 Cys Gly Cys Arg
 370

What is claimed is:

- 5 1. A method for enhancing integration of a tooth in a
mammalian tooth socket, the method comprising the step
of:
providing a therapeutically effective concentration
of a morphogen to the tooth socket surface, said
10 concentration being sufficient to induce periodontal
tissue morphogenesis in said socket.
2. The method of claim 1 wherein said step of providing a
therapeutically effective morphogen concentration to
15 said surface comprises the step of administering to
said mammal a therapeutically effective concentration
of a morphogen.
- 20 3. The method of claim 1 wherein said step of providing a
therapeutically effective morphogen concentration to
said surface comprises the step of administering to
said mammal an agent that stimulates in vivo a
therapeutically effective concentration of an
endogenous morphogen.
- 25 4. The method of claim 2 or 3 wherein said morphogen or
morphogen-stimulating agent is disposed on the surface
of the tooth root prior to implantation of said tooth
in said tooth socket.
- 30 5. The method of claim 2 or 3 wherein said morphogen or
morphogen-stimulating agent is disposed on the surface
of the tooth socket prior to implantation of said tooth
in said tooth socket.

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6. The method of claim 4 wherein said tooth root surface is partially demineralized.
- 5 7. The method of claim 5 wherein said tooth root surface is partially demineralized.
8. The method of claim 1 wherein said tooth is an implanted tooth.
- 10 9. The method of claim 1 wherein said tooth is a prosthetic tooth.
10. The method of claim 9 wherein said prosthetic tooth is an allogenic or autologous tooth.
- 15 11. The method of claim 1 wherein said therapeutically effective concentration is sufficient to induce differentiation and proliferation of cementoblasts or periodontoblasts.
- 20 12. The method of claim 1 wherein said therapeutically effective concentration is sufficient to induce formation of periodontal ligament or cementum.
- 25 13. The method of claim 2 or 3 wherein said morphogen or morphogen stimulating agent is administered to said mammal dispersed in an acellular matrix material.
- 30 14. The method of claim 13 wherein said matrix material is derived from dentin, periodontal ligament, bone, or cementum tissue.
- 35 15. A method for regenerating periodontal tissue in a mammalian tooth socket, the method comprising the step of:

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providing to the locus of the tooth socket a therapeutically effective concentration of a morphogen sufficient to induce formation of periodontal ligament or cementum.

5

16. A method for inhibiting the tissue damage associated with periodontal disease, the method comprising the step of:

10 providing a therapeutically effective concentration of a morphogen to the periodontal tissue at risk of damage.

15 17. A method for inhibiting periodontal tissue loss in a mammal, the method comprising the step of providing a therapeutically effective concentration of a morphogen to an implanted tooth or tooth socket surface, said concentration being sufficient to induce regeneration of lost or damaged periodontium.

20 18. The method of claim 1, 15, 16 or 17 wherein said therapeutic morphogen concentration is less than about 50 μ g.

25 19. The method of claim 18 wherein said therapeutic morphogen concentration is less than about 25 μ g.

30 20. The method of claim 1, 15, 16 or 17 wherein said therapeutically effective concentration is sufficient to induce formation of periodontal ligament or cementum.

35 21. The method of claim 1, 15, 16 or 17 wherein said therapeutic morphogen concentration is sufficient to induce proliferation and differentiation of cementoblasts or periodontoblasts.

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22. The method of claim 15, 16 or 17 wherein said morphogen is provided to said tissue by administering to said mammal a therapeutically effective concentration of a morphogen.
- 5
23. The method of claim 15, 16 or 17 wherein said morphogen is provided to said tissue by administering to said mammal an agent that stimulates in vivo a therapeutically effective concentration of an
- 10 endogenous morphogen.
24. A method for preparing a tooth for implantation in a mammalian tooth socket, said socket being significantly reduced in viable periodontal tissue, the method
- 15 comprising the steps of:
- (a) disposing a therapeutically effective concentration of a morphogen about the exterior surface of a tooth root to be implanted;
- (b) preparing a tooth socket to receive said tooth; and
- 20 (c) implanting said tooth in said socket.
25. The method of claim 24 comprising the additional step of partially demineralized the tooth root surface before disposing said morphogen on said surface.
- 25
26. A method for preparing a tooth socket to receive a tooth, said tooth socket being significantly reduced in viable periodontal tissue, the method comprising the steps of:
- 30 (a) preparing the tooth socket to receive a tooth;
- (b) disposing on the tooth socket surface a therapeutically effective concentration of a morphogen; and
- (c) implanting said tooth in said prepared socket.
- 35

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27. The method of claim 26 wherein said tooth root surface is partially demineralized before implantation.
28. The method of claim 1, 15, 16, 17, 24 or 26 wherein
5 said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).
- 10 29. The method of claim 28 wherein said morphogen comprises an amino acid sequence sharing a last 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, BMP3(fx), BMP5(fx), BMP6(fx),
15 Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).
30. The method of claim 1, 15, 16, 17, 24 or 26 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence
20 defined by residues 43-139 of Seq. ID No. 5 (hOP1).
31. The method of claim 30 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by
25 residues 43-139 of Seq. ID No. 5 (hOP1).
32. The method of claim 31 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species
30 variants thereof.
33. The method of claim 1, 15, 16, 17, 24 or 26 wherein said morphogen comprises an amino acid sequence defined by Generic Sequences 1, 2, 3, 4, 5 or 6 (Seq. ID
35 Nos. 1, 2, 3, 4, 30 or 31).

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34. The method of claim 1, 15, 16, 17, 24 or 26 wherein said morphogen comprises an amino acid sequence defined by OPX (Seq. ID No. 29).
- 5 35. A composition for inhibiting periodontal tissue loss in a mammal, said composition comprising a therapeutic concentration of a morphogen in association with a symptom alleviating cofactor.
- 10 36. The composition of claim 35 wherein said therapeutically effective concentration is sufficient to induce periodontal tissue morphogenesis.
- 15 37. The composition of claim 35 wherein said therapeutically effective concentration is sufficient to enhance integration of an implanted tooth in a tooth socket.
- 20 38. The composition of claim 35 wherein said cofactor comprises an antibiotic.
39. The composition of claim 35 wherein said cofactor is an antiseptic.
- 25 40. The composition of claim 35 wherein said cofactor comprises an analgesic or anesthetic.
41. The composition of claim 38 wherein said cofactor comprises tetracycline.
- 30 42. The composition of claim 35 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).
- 35

43. The composition of claim 42 wherein said morphogen comprises an amino acid sequence sharing a last 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, BMP3(fx),
5 BMP5(fx), BMP6(fx), Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).
44. The composition of claim 43 wherein said morphogen comprises an amino acid sequence having greater than
10 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).
45. The composition of claim 44 wherein said morphogen comprises an amino acid sequence having greater than
15 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).
46. The composition of claim 45 wherein said morphogen comprises an amino acid sequence defined by residues
20 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.
47. The composition of claim 46 wherein said morphogen comprises an amino acid sequence defined by Generic
25 Sequences 1, 2, 3, 4, 5 or 6 (Seq. ID Nos. 1, 2, 3, 4, 30 or 31).
48. The composition of claim 47 wherein said morphogen comprises an amino acid sequence defined by OPX (Seq.
30 ID No. 29).
49. The composition of claim 35 wherein said morphogen is dispersed in an acellular matrix.

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50. The composition of claim 49 wherein said acellular matrix is derived from dentin, bone, periodontal ligament or cementum tissue.
- 5 51. The composition of claim 35 wherein said composition comprises a solution of high viscosity.
52. The method of claim 1, 15, 16 or 17 wherein said morphogen species provided comprises the pro form.
- 10 53. The method of claim 32 wherein said morphogen species provided comprises the pro form.
54. The method of claim 53 wherein said morphogen comprises an amino acid sequence defined by residues 30-431 of Seq. ID No. 16 (hOP1), including allelic and species variants thereof.
- 15 55. The composition of claim 35 wherein said morphogen species provided comprises the pro form.
- 20 56. The composition of claim 46 wherein said morphogen species provided comprises the pro form.
- 25 57. The composition of claim 55 wherein said morphogen comprises an amino acid sequence defined by residues 30-431 of Seq. ID No. 16 (hOP-1), including allelic and species variants thereof.
- 30 58. The method of claim 3 or 23 wherein said agent stimulates expression of a morphogen in a tissue other than periodontal, dentin, or alveolar bone.

59. The use of a morphogen in the manufacture of a pharmaceutical to enhance the integration of a tooth in a tooth socket.
- 5
60. The use of a morphogen in the manufacture of a pharmaceutical to regenerate periodontal tissue or to inhibit periodontal tissue loss or the tissue damage associated with periodontal disease.
- 10
61. The use according to 59 or 60 wherein said morphogen comprises an amino acid sequence sharing at least 70% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).
- 15
62. The use according to claim 61 wherein said morphogen comprises an amino acid sequence sharing a least 80% homology with one of the sequences selected from the group consisting of: OP-1, OP-2, CBMP2, BMP3(fx), BMP5(fx), BMP6(fx), Vgl(fx), Vgr(fx), DPP(fx), GDF-1(fx) and 60A(fx).
- 20
63. The use according to claim 59 or 60 wherein said morphogen comprises an amino acid sequence having greater than 60% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).
- 25
64. The use according to claim 63 wherein said morphogen comprises an amino acid sequence having greater than 65% amino acid identity with the sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1).
- 30

65. The use according to claim 63 wherein said morphogen comprises an amino acid sequence defined by residues 43-139 of Seq. ID No. 5 (hOP1), including allelic and species variants thereof.
- 5
66. The use according to claim 59 or 60 wherein said morphogen comprises an amino acid sequence defined by Generic Sequences 1, 2, 3, 4, 5 or 6 (Seq. ID Nos. 1, 2, 3, 4, 30 or 31).
- 10
67. The use according to claim 59 or 60 wherein said morphogen comprises an amino acid sequence defined by OPX (Seq. ID No. 29).
- 15
68. The invention of claim 1, 15, 16, 17, 35, 59 or 60 wherein said morphogen comprises a polypeptide chain encoded by a nucleic acid that hybridizes under stringent conditions with the DNA sequence defined by nucleotides 1036-1341 of Seq. ID No. 16 or nucleotides 1390-1695 of Seq. ID No. 20.
- 20
69. The invention of claim 1, 15, 16, 17, 35, 59 or 60 wherein said morphogen comprises a dimeric protein species complexed with a peptide comprising a pro region of a member of the morphogen family, or an allelic, species or other sequence variant thereof.
- 25
70. The invention of claim 69 wherein said dimeric morphogen species is noncovalently complexed with said peptide.
- 30
71. The invention of claim 69 wherein said dimeric morphogen species is complexed with two said peptides.

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72. The invention of claim 69 wherein said peptide comprises at least the first 18 amino acids of a sequence defining said pro region.
- 5 73. The invention of claim 72 wherein said peptides comprises the full length form of said pro region.
74. The invention of claim 69 wherein said peptide comprises a nucleic acid that hybridizes under
10 stringent hybridization conditions with a DNA defined by nucleotides 136-192 of Seq. ID No. 16, or nucleotides 157-211 of Seq. ID No. 20.
75. The invention of claim 69 wherein said complex is
15 further stabilized by exposure to a basic amino acid, a detergent or a carrier protein.

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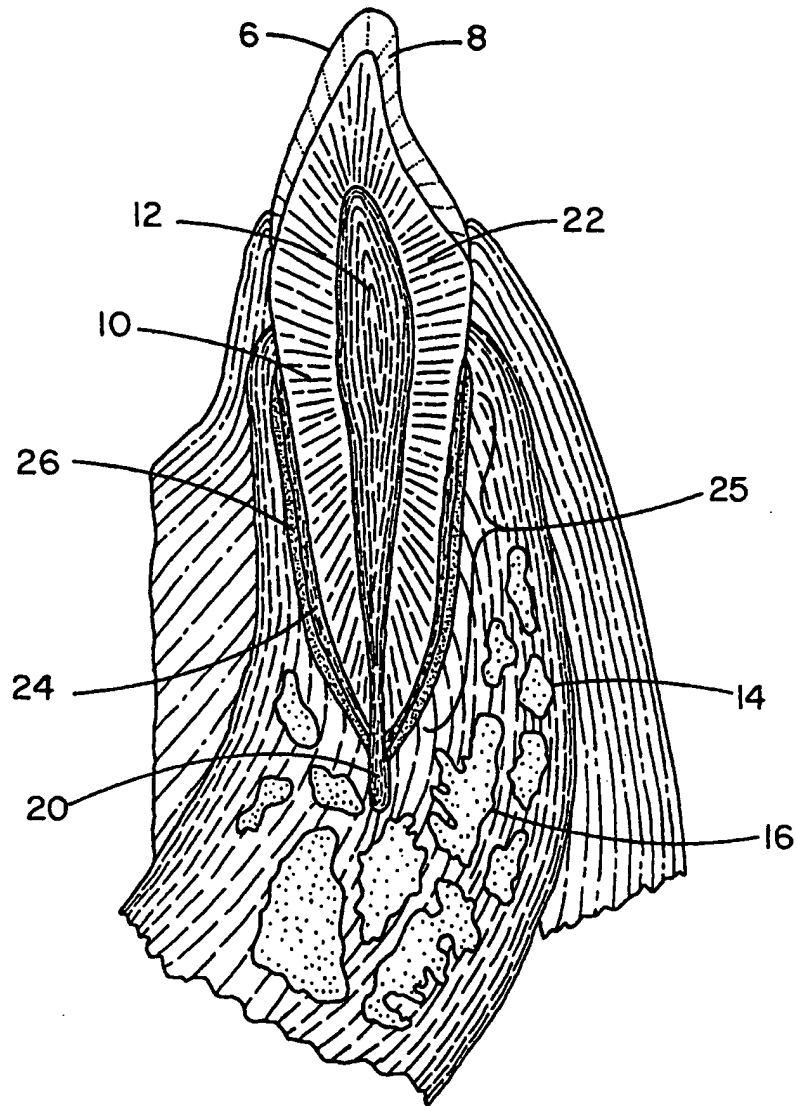


Fig.1

SUBSTITUTE SHEET

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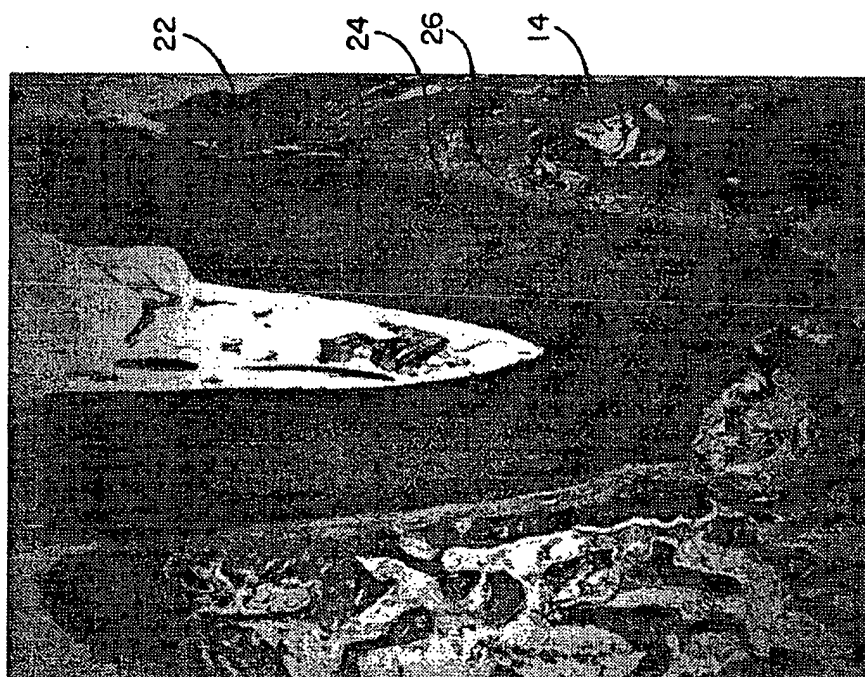


Fig. 2B

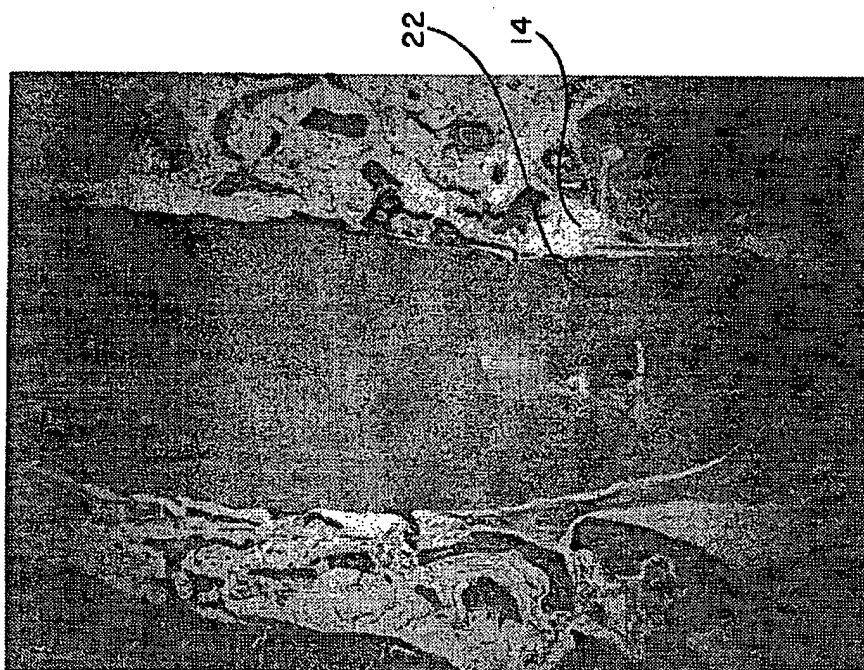


Fig. 2A

SUBSTITUTE SHEET

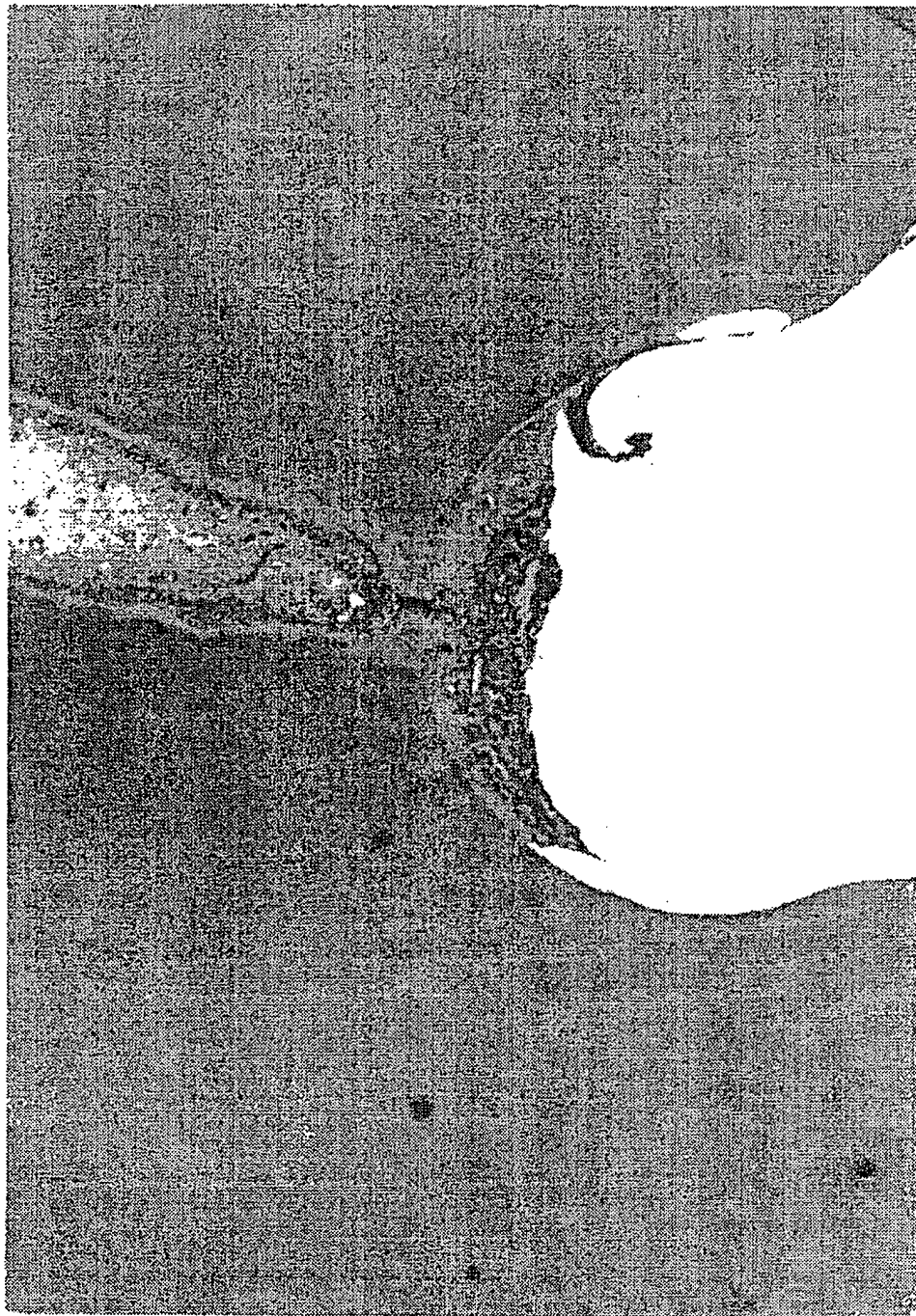


Fig. 3A

SUBSTITUTE SHEET

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Fig. 3B

SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 93/08742

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 A61K6/00 A61L27/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 A61K A61L C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO,A,92 15323 (CREATIVE BIOMOLECULES) 17 September 1992 cited in the application see page 7, line 1 - line 8 see page 13, line 5 - page 14, line 11 see claims; tables ---	1-75
X	WO,A,88 00205 (GENETICS INSTITUTE) 14 January 1988 see claims; tables see page 9, line 7 see page 9, paragraph 2 see page 10, last paragraph - page 11, paragraph 1 --- -/--	1-29, 35-37, 49,59-62

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

17 December 1993

Date of mailing of the international search report

29.12.93

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+ 31-70) 340-3016

Authorized officer

Cousins-Van Steen,G

INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/US 93/08742

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP,A,0 495 284 (OSTEOTECH, INC.) 22 July 1992 see claims; examples see column 7, line 25 - column 9, line 42 ---	1-51, 58-67
Y	US,A,5 011 691 (H. OPPERMAN) 30 April 1991 cited in the application see column 7, line 66 - column 8, line 23; claims; tables ---	1-51, 58-67
A	WO,A,90 10017 (CYTOTAXIS, INC.) 7 September 1990 -----	

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 93/08742

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: X
because they relate to subject matter not required to be searched by this Authority, namely:
REMARK: Although claims 1-34, 52-54, 58 and 68-75 (partly) are directed to a method of treatment of the human body the search has been carried out and based on the alleged effects of the composition.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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Intern. Application No

PCT/US 93/08742

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